



Divisional Application Transmittal

Customer No. 20350
TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
(415) 576-0200

ASSISTANT COMMISSIONER FOR PATENTS
BOX PATENT APPLICATION
Washington, D.C. 20231

Attorney Docket No. 15280-259120US

Client Ref No. E-002-96/2

"Express Mail" Label No. EL623999082US

Date of Deposit: October 5, 2000



Sir:

Transmitted herewith for filing under 37 CFR 1.53(b) is the

- ☐ patent application of
- ☐ continuation patent application of
- ☒ divisional patent application of
- ☐ continuation-in-part patent application of

Inventor(s)/Applicant Identifier: Ira Pastan, et al.

For: MESOTHELIN, A DIFFERENTIATION ANTIGEN PRESENT ON MESOTHELIUM, MESOTHELIOMAS AND OVARIAN CANCERS AND METHODS AND KITS FOR TARGETING THE ANTIGEN

- ☒ This application claims priority from each of the following Application Nos./filing dates:
60/010,166 filed January 5, 1996; PCT/US97/00024 filed January 3, 1997; 08/776,271 filed January 12, 1998; and 09/215,035 filed December 17, 1998, the disclosure(s) of which is (are) incorporated by reference.

Enclosed are:

- ☒ A Preliminary Amendment.
- ☒ An Information Disclosure Statement, PTO-1449, and copy of PCT Search Report (from corresponding PCT case).
- ☒ A copy of a Communication Under 37 CFR §§1.821-1.825 and Preliminary Amendment and Sequence Listing (as filed in parent case).
- ☒ 63 page(s) of specification
- ☒ 4 page(s) of claims
- ☒ 1 page of Abstract
- ☒ Letter to the Official Draftsperson.
- ☒ 3 sheet(s) of ☒ formal ☐ informal drawing(s).
- ☒ An assignment of the invention to The Government of the United States of America as represented by the Secretary of the Department of Health and Human Services is on file and is still proper and desired.
- ☒ Copies of ☒ signed Declarations & Powers of Attorney (from parent case, application number 08/776,271 filed January 12, 1998).

Applicant requests deferral of the filing fee until submission of the Missing Parts of Application.

DO NOT CHARGE THE FILING FEE AT THIS TIME.

[Telephone:
(415) 576-0200

Facsimile:
(415) 576-0300

Respectfully submitted,
TOWNSEND and TOWNSEND and CREW LLP

Laurence J. Hyman
Reg No.: 35,551
Attorneys for Applicant

"Express Mail" Label No. EL623999082US

Date of Deposit: October 5, 2000

PATENT

Attorney Docket No.: 15280-259120US

Client Reference No.: E-002-96/2

BOX PATENT APPLICATION

Assistant Commissioner for Patents

Washington, D.C. 20231

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Ira Pastan, *et al.*

Examiner: A. Holleran

Art Unit: 1642

Divisional of Application No.: 09/215,035

PRELIMINARY AMENDMENT

Filed: December 17, 1998

For: MESOTHELIN, A
DIFFERENTIATION ANTIGEN
PRESENT ON MESOTHELIUM,
MESOTHELIOMAS AND OVARIAN
CANCERS AND METHODS AND KITS
FOR TARGETING THE ANTIGEN

BOX PATENT APPLICATION

Assistant Commissioner for Patents

Washington, D.C. 20231

Sir:

Prior to examination of the above-referenced application, please enter the following amendments and remarks.

IN THE SPECIFICATION:

On page 1, under "CROSS REFERENCE TO RELATED APPLICATIONS," please delete the entirety of the sentence and substitute in its place:

--This application is a divisional of U.S. Application No. 09/215,035, filed December 17, 1998, which is a divisional of U.S. Application No. 08/776,271, filed January 12, 1998, which is a national stage filing under 35 U.S.C. § 371 of PCT/US97/00224, filed January 3, 1997, which claims priority from U.S. Provisional Application No. 60/010,166, filed January 5, 1996, all of which are hereby incorporated by reference.--

IN THE CLAIMS:

Please cancel claims 1-16 and 18. Claim 17 is the only pending claim.

REMARKS

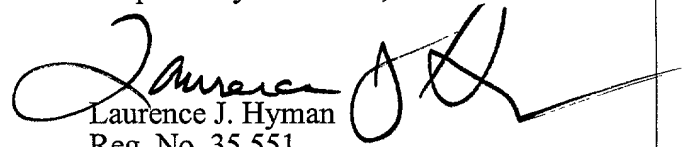
The amendment to the specification updates the priority information to reflect the filing of this divisional application. The amendment to the claims cancels claims 1-16 and 18 examined in parent case 08/776,271, filed January 12, 1998.

CONCLUSION

In view of the foregoing, Applicants believe the claim now pending in this Application is in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, he or she is invited to telephone the undersigned at 415-576-0200.

Respectfully submitted,


Laurence J. Hyman
Reg. No. 35,551

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
LJH:kad
SF 1143567 v1

00564593-100000

"Express Mail" Label No. E-157572370945
Date of Deposit Dec. 17, 1998

PATENT
Attorney Docket No.: 015280-259110US

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Assistant Commissioner for Patents
Washington, D.C. 20231

By: Samuel Butth

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

PASTAN and CHANG

Application No.: Not yet assigned

Filed: Herewith

For: MESOTHELIN, A
DIFFERENTIATION ANTIGEN
PRESENT ON MESOTHELIUM,
MESOTHELIOMAS AND OVARIAN
CANCERS AND METHODS AND KITS
FOR TARGETING THE ANTIGEN

Examiner: Not yet assigned

Art Unit: Not yet assigned

COMMUNICATION UNDER

37 C.F.R. §§ 1.821-1.825

AND

PRELIMINARY AMENDMENT

Box SEQUENCE
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In order to comply with Requirements for Patent Applications Containing
Nucleotide Sequence and/or Amino Acid Sequence Disclosures, 37 C.F.R. §§ 1.821-1.825,
Applicants submit herewith the required paper copy and computer readable copy of the
Sequence Listing. Please amend the specification in adherence with 37 C.F.R. §§ 1.821-
1.825 as follows.

Prior to examination of the above-referenced application, please enter the
following amendments and remarks.

IN THE SPECIFICATION:

On page 3, line 17, replace "SEQ. I.D. NO. 2" with --SEQ ID NO:2--.

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On page 3, line 19, replace "SEQ. I.D. NO. 2" with --SEQ ID NO:2--.

On page 3, line 33, replace "SEQ. I.D. NO. 1" with --SEQ ID NO:1--.

On page 3, line 37, replace "SEQ. I.D. NO. 1" with --SEQ ID NO:1--.

On page 8, line 2, insert --(SEQ ID NO:1)-- after "Nucleotide sequence".

On page 8, line 3, insert --(SEQ ID NO:2)-- after "acid sequence".

On page 9, lines 5 and 6, replace "Sequence I.D. Nos. 1 and 2" with --SEQ ID NOS:1 and 2--.

On page 14, lines 19 and 20, replace "SEQ. I.D. NOS. 1" with --SEQ ID NOS:1 and 2--.

On page 15, lines 5 and 6, replace "SEQ. I.D. NO. 1" with --SEQ ID NO:1--.

On page 20, line 36, replace "SEQ. I.D. NO. 2" with --SEQ ID NO:2--.

On page 20, line 38, replace "SEQ. I.D. NO. 2" with --SEQ ID NO:2--.

On page 21, line 2, replace "SEQ. I.D. NO. 2" with --SEQ ID NO:2--.

On page 21, lines 5 and 6, replace "SEQ. I.D. NO. 2" with --SEQ ID NO:2--.

On page 29, line 9, replace "[SEQ. I.D. NO. 3]" with --(SEQ ID NO:3)--.

On page 29, lines 23 and 24, replace "[SEQ. I.D. NO. 4]" with --(SEQ ID NO:4)--.

On page 29, line 24, replace "[SEQ. I.D. NO. 5]" with --(SEQ ID NO:5)--.

On page 29, lines 24 and 25, replace "[SEQ. I.D. NO. 6]" with --(SEQ ID NO:6)--.

On page 29, line 25, replace "[SEQ. I.D. NO. 7]" with --(SEQ ID NO:7)--.

On page 30, line 5, replace "[SEQ. I.D. NO. 4]" with --(SEQ ID NO:4)--.

On page 30, lines 5 and 6, replace "[SEQ. I.D. NO. 7]" with --(SEQ ID NO:7)--.

On page 30, line 6, replace "[SEQ. I.D. NO. 6]" with --(SEQ ID NO:6)--.

On page 56, line 5, insert --(SEQ ID NO:8)-- after "RPRFRR".

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Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 8, at the end of the application.

IN THE CLAIMS:

In claim 1, line 4, replace "SEQ. I.D. NO. 2" with --SEQ ID NO:2--.

In claim 1, line 6, replace "SEQ. I.D. NO. 2" with --SEQ ID NO:2--.

In claim 6, line 3, replace "SEQ. I.D. NO. 2" with --SEQ ID NO:2--.

In claim 6, line 5, replace "SEQ. I.D. NO. 2" with --SEQ ID NO:2--.

In claim 10, line 3, replace "SEQ. I.D. NO. 2" with --SEQ ID NO:2--.

In claim 10, line 5, replace "SEQ. I.D. NO. 2" with --SEQ ID NO:2--.


REMARKS

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-8, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,


Susan K. Faris
Reg. No. 41,739

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
SKF:dmw

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5 MESOTHELIN, A DIFFERENTIATION ANTIGEN PRESENT ON
MESOTHELIUM, MESOTHELIOMAS AND OVARIAN CANCERS
AND METHODS AND KITS FOR TARGETING THE ANTIGEN

FIELD OF THE INVENTION

10 This invention relates to the identification of a
specific antigen found on tumor cells, particularly
mesotheliomas and ovarian tumor cells and, *inter alia*, methods
and compositions for targeting cells bearing the antigen.

BACKGROUND OF THE INVENTION

15 Monoclonal antibodies are currently being used to
diagnose and treat cancer (Mach, J., et al., *Current Opinion*
Immunol. B, 685-693 (1991); Grossbard, M.L., et al., *Blood* 80
(4):863-878 (1992)). To be useful for therapy, the antibody
20 should recognize an antigen that is present in large amounts
on the cancer cells and in negligible amounts in normal cells.
Alternatively the antigen can be present in substantial
amounts on normal cells, if the normal cells are not
components of an essential organ. This approach has been
useful in developing new treatments for leukemias and
25 lymphomas. Several differentiation antigens have been
identified on lymphomas and leukemias which are good targets
for immunotherapy, because they are not present on the stem
cells which give rise to differentiated lymphocytes
(Grossbard, M.L., et al., *Blood* 80 (4):863-878 (1992)). Thus,
30 normal lymphocytes that are killed by immunotherapy can be
regenerated. Some examples of lymphocyte antigens of this
type are CD19, CD22, CD25 and CD30 (Grossbard, M.L., et al.,
Blood 80 (4):863-878 (1992); Engert, A., et al., *Cancer*
Research 50, 84-88 (1990)). Clearly, it would be very useful
35 to have antibodies that recognize differentiation antigens on
solid tumors, but only a small number of these are available.
One reason contributing to the paucity of such antibodies is
that efforts to identify differentiation antigens on various
types of epithelial cells have been relatively modest compared

with the intense efforts made to identify differentiation antigens on cells of the hematopoietic system.

Ovarian cancer represents one of the diseases which could be treated by immunotherapy, because the ovaries are always removed during surgery for this disease and reactivity with normal ovarian tissue is not a problem. Several antibodies that recognize differentiation antigens on ovarian cancer cells have been generated. One of these is OC125 that recognizes the CA125 antigen (Bast, R., et al., *N. Eng. J. Med.* 309, 883-887 (1983)). CA125 is a high molecular weight glycoprotein that is shed by ovarian cancer cells and has been useful in the diagnosis of ovarian cancer. However, antibodies to CA125 are not useful for immunotherapy because the CA125 antigen is shed into the blood stream (Bast, R., et al., *N. Eng. J. Med.* 309, 883-887 (1983)). Another is MOV18 which recognizes the folate binding protein. This protein is abundant in ovarian cancers as well as in some other tumors. Unfortunately, this protein is also abundantly expressed in kidney (Campbell, I.G., et al., *Cancer Res.* 51, 5329-5338 (1991)). An antibody we previously isolated and termed MAb K1 reacts with many ovarian cancers and many mesotheliomas. Like OC125, the antibody also reacts with normal mesothelial cells, but it does not react with other cell types except for weak reactivity with some cells in the trachea (Chang, K., et al., *Int. J. Cancer* 50, 373-381 (1992); Chang, K., et al., *Cancer Res.* 52, 181-186 (1992), see also U.S. Patent 5,320,956). The antigen recognized by MAb K1 appears to be a differentiation antigen present on mesothelium and is expressed on cancers derived from mesothelium such as epithelioid type mesotheliomas as well as on most ovarian cancers. Thus immunotherapy directed at the CAK1 antigen should take into account the potential risk of damaging normal mesothelial cells and perhaps cells of the trachea (Chang, K., et al., *Int. J. Cancer* 50, 373-381 (1992); Chang, K., et al., *Cancer Res.* 52, 181-186 (1992); Chang, K., et al., *Int. J. Cancer* 51, 548-554 (1992); Chang, K., et al., *Am. J. Surg. Pathol.* 16, 259-268 (1992)).

Using the ovarian cancer cell line OVCAR-3 as well as HeLa cells, the antigen has been shown to be an approximately 40 kD glycoprotein that is attached to the cell surface by phosphatidylinositol. The protein is released when cells are treated with phosphatidylinositol specific phospholipase C (Chang, K., et al., *Cancer Res.* **52**, 181-186 (1992)). We had previously attempted to clone a cDNA encoding the CAK1 antigen but instead cloned cDNAs encoding two different intracellular proteins which also react with MAb K1 (Chang, K., and Pastan, I., *Int. J. Cancer* **57**, 90-97 (1994)). Neither of these is the cell surface membrane antigen recognized by MAb K1.

SUMMARY OF THE INVENTION

The present invention provides uses for isolated polypeptides comprising at least 10 contiguous amino acids from the polypeptide sequence of SEQ. I.D. NO. 2, wherein the polypeptide binds to antisera raised against the full-length polypeptide of SEQ. I.D. NO. 2 as an immunogen, which has been fully immunoadsorbed with a 40 kD polypeptide attached to the cell surface of OVCAR-3 and HeLa cells (the K1 antigen). Full-length polypeptides of the invention are typically about 69 kD in size, although they are larger when glycosylated or incorporated into a construct such as an eukaryotic expression vector. The polypeptides of the present invention may be present in several forms, including isolated naturally occurring endoproteolytic polypeptides, recombinantly produced polypeptides, and as portions of recombinant polypeptides such as fusion proteins.

The present invention also provides uses for isolated nucleic acids which encode the polypeptides described above. Exemplary nucleic acids include those described in SEQ. I.D. NO. 1. In preferred embodiments, the nucleic acid is part of a recombinant vector such as a plasmid or virus or may be used as a probe to detect for the antigen. In preferred embodiments, the nucleic acid selectively hybridizes to the nucleic acid of SEQ. I.D. NO. 1. The nucleic acid sequence may encode, e.g., a mesothelin polypeptide with

complete sequence identity to a naturally occurring mesothelin protein. The nucleic acid may also encode a mesothelin polypeptide which is not identical to a naturally occurring mesothelin polypeptide, such as a fusion protein, or a
5 genetically engineered mutant mesothelin protein which retains the bases critical for protein function or immunogenicity as described herein.

Recombinant cells which comprise a nucleic acid of the present invention are also provided, including eukaryotic
10 and prokaryotic cells. The present invention also provides antibodies which bind specifically to the polypeptides of the present invention.

The invention further provides methods for targeting and/or inhibiting the growth of cells bearing mesothelin;
15 methods for detecting the antigen and its expression level as an indication of the presence of tumor cells; and kits for such detection.

DEFINITIONS

Unless defined otherwise, all technical and
20 scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the
25 practice or testing of the present invention, the preferred methods and materials are described. For purposes of the present invention, the following terms are defined below.

The term "antibody" as used herein, includes various forms of modified or altered antibodies, such as an intact
30 immunoglobulin, various fragments such as an Fv fragment, an Fv fragment containing only the light and heavy chain variable regions, an Fv fragment linked by a disulfide bond (Brinkmann, et al. *Proc. Natl. Acad. Sci. USA*, 90: 547-551 (1993)), an Fab or (Fab)₂ fragment containing the variable regions and parts
35 of the constant regions, a single-chain antibody and the like (Bird et al., *Science* 242: 424-426 (1988); Huston et al., *Proc. Nat. Acad. Sci. USA* 85: 5879-5883 (1988)). The antibody may be of animal (especially mouse or rat) or human origin or

may be chimeric (Morrison *et al.*, *Proc Nat. Acad. Sci. USA* 81: 6851-6855 (1984)) or humanized (Jones *et al.*, *Nature* 321: 522-525 (1986), and published UK patent application #8707252).

The term "immunoassay" is an assay that utilizes an antibody to specifically bind an analyte or antigen. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the analyte.

The terms "isolated," "purified," or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state.

The term "nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides.

The term "nucleic acid probe" refers to a molecule which binds to a specific sequence or subsequence of a nucleic acid. A probe is preferably a nucleic acid which binds through complementary base pairing to the full sequence or to a subsequence of a target nucleic acid. It will be understood by one of skill in the art that probes may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. The probes are preferably directly labelled as with isotopes, chromophores, lumiphores, chromogens, or indirectly labelled such as with biotin to which a streptavidin complex may later bind. By assaying for the presence or absence of the probe, one can detect the presence or absence of the select sequence or subsequence.

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

The term "recombinant" when used with reference to a cell indicates that the cell encodes a DNA whose origin is exogenous to the cell-type. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell.

The term "identical" in the context of two nucleic acids or polypeptide sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (U.S.A.)* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

The term "substantial identity" or "substantial similarity" in the context of a polypeptide indicates that a polypeptide comprises a sequence with at least 70% sequence identity to a reference sequence, or preferably 80%, or more preferably 85% sequence identity to the reference sequence, or most preferably 90% identity over a comparison window of about 10-20 amino acid residues. An indication that two polypeptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a polypeptide is substantially identical to a second polypeptide, for example, where the two peptides differ only by a conservative substitution.

An indication that two nucleic acid sequences are substantially identical is that the polypeptide which the first nucleic acid encodes is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions. Stringent conditions are sequence dependent and are different under

different environmental parameters. Generally, stringent conditions are selected to be about 5° C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. However, nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

The phrases "specifically binds to a protein" or "specifically hybridizes to" or "specifically immunoreactive with", when referring to an antibody refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind preferentially to a particular protein and do not bind in a significant amount to other proteins present in the sample. Specific binding to a protein under such conditions requires an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Nucleotide sequence and deduced amino acid sequence of the CAK1-9 cDNA. The nucleotide sequence (upper line) and the deduced amino acid sequence (lower line) of the CAK1 cDNA is listed with nucleotide numbers at left. The translation of CAK1 starts at nucleotides 100-102 (ATG) and terminates at 1986-88 (TGA). The putative signal peptide is underlined and a typical hydrophobic sequence for GPI anchorage is double-underlined. A likely furin cleavage site RPRFRR is underlined and the cleavage site shown by an arrow. There are four potential N-linked glycosylation sites (in bold letters). A variant polyadenylation signal (AGTAAA) is present 22 base pairs upstream from the polyadenylation tail. The original p6-1 cDNA sequence spans nucleotides 721 to 2138.

Figure 2: Different forms of the CAK1 tumor antigen. S.P. = putative signal peptide; H.P. = GPI anchorage dependent hydrophobic peptide; CHO = carbohydrates; M = membrane, AA = amino acids.

DETAILED DESCRIPTION

This invention relates to the discovery of an antigen, referred to herein as mesothelin, found on mesothelium, mesotheliomas, ovarian cancer cells and some squamous cell carcinomas. Previously, an antibody designated monoclonal antibody K1 was described which reacts with an antigen found on OVCAR-3 cells (from a human ovarian tumor cell line) having a molecular weight of 40 kD (kilodaltons). See, e.g. U.S. Patent No. 5,320,956. The antigen described and claimed here was unexpectedly obtained during an attempt to clone and sequence the K1 antigen. Mesothelin in its full-length form has an apparent molecular weight of about 69 kD and appears to be the precursor protein for the 40 kD K1 antigen. The K1 antigen itself proved difficult to clone and our first attempts resulted in the cloning of two different intracellular proteins as mentioned above (see Chang & Pastan, *Int. J. Cancer, supra*). Though the existence of the K1 antigen was known, its cDNA was not routine to clone. First, we were not able to obtain sufficient amounts of it to clone.

The methods used here were more laborious, but successful because unbeknownst to us the K1 antigen was derived from a larger molecule that we did not know existed. The DNA sequence and corresponding amino acid sequence for full-length mesothelin are set out in Figure 1 and in Sequence I.D. Nos. 1 and 2, respectively.

Reference to mesothelin herein refers to both the isolated full-length polypeptide and isolated polypeptide fragments of at least 10 contiguous amino acids from the full-length sequence wherein the fragment binds to antisera raised against the full-length polypeptide, which has been fully immunosorbed with the 40 kD K1 antigen.

Mesothelin, as described here represents an antigen which is found on mesothelium, mesotheliomas, ovarian cancers and some squamous cell carcinomas. We have designated this antigen mesothelin to reflect its presence on mesothelial cells. The full-length cDNA for mesothelin is 2138 bp in length and contains an open reading frame of 1884 bp. The protein it encodes contains 628 amino acids with a calculated molecular weight of about 69000 daltons in its full-length form.

The protein contains four potential N-linked glycosylation sites N-X-S or N-X-T that are shown in bold letters in Figure 1. A typical signal sequence is not present at the amino terminus. However, a short hydrophobic segment is located 15 amino acids from the first methionine (Fig. 1). This sequence might function as a signal sequence for membrane insertion, because the protein is found on the cell surface and is inserted into microsomes during cell free translation. Also present is a putative proteolytic processing site, RPRFRR, beginning at amino acid 293 (Fig. 1). This site is recognized by furin, a protease important in the processing of several membrane proteins as well as in the activation of *Pseudomonas* and diphtheria toxins (Chiron, M.F., et al., *J.B.C.* 269(27):18169-18176 (1994)).

The 40 kD form ("K1") appears to be derived from a 69 kD precursor by several processing steps. These are summarized in Fig. 2. Initially, mesothelin is made as a 69

kD polypeptide with a hydrophobic tail which is probably removed and replaced by phosphatidylinositol (Chang, K., et al., *Cancer Res.* **52**, 181-186 (1992)). After glycosylation at one or more of its four putative N-linked glycosylation sites, it is cleaved by a protease to yield higher molecular weight forms, the 40 kD fragment (or doublet) found on the surface of OVCAR-3 cells and a smaller (~31 kD) fragment. The latter could be released into the medium and/or further degraded. We found that the amino terminal fragment was detected in the medium of OVCAR-3 cells.

Mesothelin is one of many proteins and glycoproteins that are attached to the cell surface by phosphatidylinositol. Several functions have been ascribed to these molecules. Some are receptors involved in cell signaling; others are involved in cellular recognition and/or adhesion (Dustin, M.L., et al., *Nature* **329**, 846-848 (1987); Stiernberg, J., et al., *J. Immunol.* **38**, 3877-3884 (1987)). GPI linked proteins may interact with tyrosine kinases (Stefanova, I., et al., *Science* **254**, 1016-1019 (1991); Pandey, A., et al., *Science* **268**, 567-569 (1995)). Antibodies to mesothelin would be useful in inhibiting the spread or implantation of ovarian cancer cells into the peritoneal wall that sometimes occurs, for example, during ovarian cancer surgery. Without intending to be bound by theory, it is our belief that mesothelin is likely responsible for the adhesion and implantation of ovarian carcinoma cells that frequently occurs throughout the peritoneal cavity or the adhesion of tumor cells in the thoracic cavity. Mesothelin plays a role in adhesion since mesothelin transfectants are more slowly removed from culture dishes than non-transfected cells. Mesothelial cells are extremely flat and regulate the traffic of molecules and cells in and out of the peritoneal or thoracic cavity.

Mesothelin is very abundant in normal mesothelial cells from which malignant mesotheliomas and ovarian cystadenocarcinomas are derived. These two types of tumors share a unique biological characteristic that distinguishes them from other solid tumors. In the early stages, both types of tumors spread aggressively throughout the peritoneal (or

thoracic) cavity and invade locally but do not metastasize distally through lymphatics or the blood stream. In fact, many patients succumb to their cancer before distant metastases develop. Mesothelin likely has a role in this process, since cells overexpressing mesothelin have altered adhesive properties and mesothelin expression is diminished in poorly differentiated ovarian cancers (Chang, K., et al., *Int. J. Cancer* 51, 548-554 (1992); Chang, K., et al., *Am. J. Surg. Pathol.* 16, 259-268 (1992)). Implantation of ovarian cancer cells through a strong adhesion mechanism may be the first step towards local invasion and distal metastasis. Thus, blocking ovarian cancer implantation will prevent invasion and metastasis as well as proliferation of the cancer cells and lead cancer cells to apoptosis and the like.

I. *Detection for Mesothelin*

The detection of mesothelin is useful as an indicator of the presence of tumor cells, particularly ovarian tumor cells or mesotheliomas. If found in serum it can be a factor indicating the presence of residual cancer cells. Tumor tissues contain various proteases which may be responsible for the cleavage of mesothelin. The amount of N-terminal fragment of mesothelin present in blood or ascitic fluid can reflect the number of residual tumor cells present. The serological detection of mesothelin may serve as a novel indicator for monitoring the process of disease. The basic principle for detection of the mesothelin proteins is to detect the protein using specific ligands that bind to mesothelin but not to other proteins or nucleic acids in a normal human cell or its environs. The ligands can be either nucleic acid or antibodies. The ligands can be naturally occurring or genetically or physically modified such as non-natural or antibody derivatives, i.e. FAB, or chimeric antibodies.

A. *Sample Collection and Processing*

Mesothelin is preferably quantified in a biological sample, such as a serum, cell, or a tissue sample derived from a patient. In a preferred embodiment, mesothelin is

quantified in samples of serum, mesothelial cells, cervical tissue or ovarian tissue with reference to a standard prepared from recombinant mesothelin.

The sample may be pretreated as necessary by dilution in an appropriate buffer solution or concentrated, if desired depending upon the assay being used. Any of a number of standard aqueous buffer solutions, employing one of a variety of buffers, such as phosphate, Tris, or the like, at physiological pH can be used.

B. *Quantification of mesothelin peptides.*

Mesothelin peptides may be detected and quantified by any of a number of means well known to those of skill in the art. These include analytic biochemical methods such as electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like, and various immunological methods such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, and the like.

C. *General Techniques - Nucleic Acid Detection*

Accepted means for conducting hybridization assays for detection are known and general overviews of the technology can be had from a review of: *Nucleic Acid Hybridization: A Practical Approach*, Ed. Hames, B.D. and Higgins, S.J., IRL Press, 1985; *Hybridization of Nucleic Acids Immobilized on Solid Supports*, Meinkoth, J. and Wahl, G.; *Analytical Biochemistry*, Vol 238, 267-284, 1984 and Innis et al., *PCR Protocols*, supra, all of which are incorporated by reference herein.

If PCR is used, for example, primers are designed to target a specific portion of the nucleic acid of the targeted agent. Preferably the primers are about 14 to about 24 nucleotides in length. From the sequence information provided herein, those of skill in the art will be able to select appropriate specific primers.

Target specific probes may be used in the nucleic acid hybridization diagnostic assays for mesothelin. The probes are specific for or complementary to the target of interest. For example, probes to one of the nucleic acid sequences in the open reading frame for mesothelin would be effective. For precise allelic differentiation, the probes should be about 14 nucleotides long and preferably about 20-30 nucleotides. For more general detection, nucleic acid probes are about 50 to about 1000 nucleotides, most preferably about 200 to about 400 nucleotides.

The detection of the mesothelin polypeptides and other aspects of the present invention may make use of techniques such as PCR, TAS, 3SR, QB amplification and cloning, to amplify a nucleic acid in a biological sample which encodes a mesothelin polypeptide for detection or for, *inter alia*, the production of probes and primer tools for detection.

The presence of mesothelin nucleic acid in a biological sample such as, for example, serum or tissue suspected to contain tumor cells, is useful, e.g., as a probe to assess the presence of mesothelin and subsequently provide evidence indicative of tumor cells.

The nucleic acids of the present invention are cloned, or amplified by *in vitro* methods, such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification system (TAS), the self-sustained sequence replication system (3SR) and the Q β replicase amplification system (QB). A wide variety of cloning and *in vitro* amplification methodologies are well-known to persons of skill. Examples of these techniques and instructions sufficient to direct persons of skill through many cloning exercises are found in Berger and Kimmel, *Guide to Molecular Cloning Techniques, Methods in Enzymology* 152 Academic Press, Inc., San Diego, CA (Berger); Sambrook et al. (1989) *Molecular Cloning - A Laboratory Manual* (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, (Sambrook et al.); *Current Protocols in Molecular Biology*, F.M. Ausubel et al., eds., Current Protocols, a joint venture

between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1994 Supplement) (Ausubel); Cashion et al., U.S. patent number 5,017,478; and Carr, European Patent No. 0,246,864. Examples of techniques sufficient to direct persons of skill through *in vitro* amplification methods are found in Berger, Sambrook, and Ausubel, as well as Mullis et al., (1987) U.S. Patent No. 4,683,202; *PCR Protocols A Guide to Methods and Applications* (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) (Innis); Arnheim & Levinson (October 1, 1990) *C&EN* 36-47; *The Journal Of NIH Research* (1991) 3, 81-94; (Kwoh et al. (1989) *Proc. Natl. Acad. Sci. USA* 86, 1173; Guatelli et al. (1990) *Proc. Natl. Acad. Sci. USA* 87, 1874; Lomell et al. (1989) *J. Clin. Chem* 35, 1826; Landegren et al., (1988) *Science* 241, 1077-1080; Van Brunt (1990) *Biotechnology* 8, 291-294; Wu and Wallace, (1989) *Gene* 4, 560; and Barringer et al. (1990) *Gene* 89, 117.

It will be readily understood by those of skill in the art and it is intended here, that when reference is made to particular sequence listings, such as SEQ. I.D. NOS. 1 and 2, such reference includes sequences which substantially correspond to its complementary sequence and those described including allowances for minor sequencing errors, single base changes, deletions, substitutions and the like, such that any such sequence variation corresponds to the nucleic acid sequence to which the relevant sequence listing relates.

D. Antibodies to Mesothelin and Antibody-Ligand Binding Assays

Antibodies (or antisera) are raised to the polypeptides of the present invention, including individual fragments thereof, both in their naturally occurring (full-length) forms and in recombinant forms. Additionally, antibodies are raised to these polypeptides in either their native configurations or in non-native configurations. Anti-idiotypic antibodies can also be generated. Many methods of making antibodies are known to persons of skill. The following discussion is presented as a general overview of the techniques available; however, one of skill will recognize that many variations upon the following methods are known.

1. Antibody Production

A number of immunogens are used to produce antibodies specifically reactive with mesothelin polypeptides. Recombinant or synthetic polypeptides of 10 amino acids in length, or greater, selected from sub-sequences of SEQ. I.D. NO. 1 are the preferred polypeptide immunogen for the production of monoclonal or polyclonal antibodies. In one class of preferred embodiments, an immunogenic peptide conjugate is also included as an immunogen. Naturally occurring polypeptides are also used either in pure or impure form. Transfected mammalian cells overexpressing recombinant mesothelin can also be used as an immunogen, either in whole intact cells or membrane preparations. These immunogens are useful for polyclonal or monoclonal antibody generation.

Recombinant polypeptides are expressed in eukaryotic or prokaryotic cells and purified using standard techniques. The polypeptide, or a synthetic version thereof, is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies can be generated for subsequent use in immunoassays to measure the presence and quantity of the polypeptide.

Methods of producing polyclonal antibodies are known to those of skill in the art. In brief, an immunogen, preferably a purified polypeptide, a polypeptide coupled to an appropriate carrier (e.g., GST, keyhole limpet hemocyanin, etc.), or a polypeptide incorporated into an immunization vector such as a recombinant vaccinia virus (see, U.S. Patent No. 4,722,848) is mixed with an adjuvant and animals are immunized with the mixture. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the polypeptide of interest. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the polypeptide is performed where desired. See, e.g., Coligan (1991) *Current Protocols in Immunology* Wiley/Greene, NY; and Harlow and Lane (1989) *Antibodies: A Laboratory Manual* Cold Spring Harbor

Press, NY, which are incorporated herein by reference, and the examples below.

Antibodies, including binding fragments and single chain recombinant versions thereof, against predetermined fragments of mesothelin polypeptides are raised by immunizing animals, e.g., with conjugates of the fragments with carrier proteins as described above. Typically, the immunogen of interest is a peptide of at least about 3 amino acids, more typically the peptide is 5 amino acids in length, preferably, the fragment is 10 amino acids in length and more preferably the fragment is 15 amino acids in length or greater. The peptides are typically coupled to a carrier protein (e.g., as a fusion protein), or are recombinantly expressed in an immunization or expression vector. Antigenic determinants on peptides to which antibodies bind are typically 3 to 10 amino acids in length.

Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies are screened for binding to normal or modified polypeptides. Specific monoclonal and polyclonal antibodies will usually bind with a K_D of at least about .1 mM, more usually at least about 50 μ M, and most preferably at least about 1 μ M or better.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies are well known and are found in, e.g., Asai, ed. *Antibodies in Cell Biology*, Academic Press, Inc., San Diego, CA; Stites et al. (eds.) *Basic and Clinical Immunology* (4th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane, *Supra*; Goding (1986) *Monoclonal Antibodies: Principles and Practice* (2d ed.) Academic Press, New York, NY; and Kohler and Milstein (1975) *Nature* 256: 495-497. The polypeptides and antibodies of the present invention are used with or without modification, and include chimeric antibodies such as humanized murine antibodies.

Other suitable techniques involve selection of libraries of recombinant antibodies in phage or similar

vectors. See, Huse et al. (1989) *Science* 246: 1275-1281; and Ward, et al. (1989) *Nature* 341: 544-546.

Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionucleotides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced. See, for example, Cabilly, U.S. Patent No. 4,816,567; and Queen et al. (1989) *Proc. Nat'l Acad. Sci. USA* 86: 10029-10033.

The antibodies of this invention are also used for affinity chromatography in isolating mesothelin polypeptides. Columns are prepared, e.g., with the antibodies linked to a solid support, e.g., particles, such as agarose, Sephadex, or the like, where a cell lysate is passed through the column, washed, and treated with increasing concentrations of a mild denaturant, whereby purified mesothelin polypeptides are released.

The antibodies can be used to screen expression libraries for particular expression products such as mammalian mesothelin. Usually the antibodies in such a procedure are labeled with a moiety allowing easy detection of presence of antigen by antibody binding.

Antibodies raised against mesothelin polypeptides can also be used to raise anti-idiotypic antibodies. These are useful for detecting or diagnosing various pathological conditions related to the presence of the respective antigens.

2. Immunoassays

A particular protein can be quantified by a variety of immunoassay methods. For a review of immunological and immunoassay procedures in general, see Stites and Terr (eds.) 1991 *Basic and Clinical Immunology* (7th ed.). Moreover, the

immunoassays of the present invention can be performed in any of several configurations, e.g., those reviewed in Maggio (ed.) (1980) *Enzyme Immunoassay* CRC Press, Boca Raton, Florida; Tijan (1985) "Practice and Theory of Enzyme Immunoassays," *Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers B.V., Amsterdam; Harlow and Lane, *supra*; Chan (ed.) (1987) *Immunoassay: A Practical Guide* Academic Press, Orlando, FL; Price and Newman (eds.) (1991) *Principles and Practice of Immunoassays* Stockton Press, NY; and Ngo (ed.) (1988) *Non-isotopic Immunoassays* Plenum Press, NY.

Immunoassays also often utilize a labeling agent to specifically bind to and label the binding complex formed by the capture agent and the analyte. The labeling agent may itself be one of the moieties comprising the antibody/analyte complex. Thus, the labeling agent may be a labeled mesothelin peptide or a labeled anti-mesothelin antibody. Alternatively, the labeling agent may be a third moiety, such as another antibody, that specifically binds to the antibody/mesothelin complex, or to a modified capture group (e.g., biotin) which is covalently linked to the mesothelin peptide or anti-mesothelin antibody.

In a preferred embodiment, the labeling agent is an antibody that specifically binds to the capture agent (anti-mesothelin). Such agents are well known to those of skill in the art, and most typically comprise labeled antibodies that specifically bind antibodies of the particular animal species from which the capture agent is derived (e.g., an anti-idiotypic antibody). Thus, for example, where the capture agent is a mouse derived anti-human mesothelin antibody, the label agent may be a goat anti-mouse IgG, i.e., an antibody specific to the constant region of the mouse antibody.

Other proteins capable of specifically binding immunoglobulin constant regions, such as streptococcal protein A or protein G are also used as the labeling agent. These proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety

of species. See, generally Kronval, et al., (1973) *J. Immunol.*, 111:1401-1406, and Akerstrom, et al., (1985) *J. Immunol.*, 135:2589-2542.

Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, preferably from about 5 minutes to about 24 hours. However, the incubation time will depend upon the assay format, analyte, volume of solution, concentrations, and the like. Usually, the assays are carried out at ambient temperature, although they can be conducted over a range of temperatures, such as 5°C to 45°C.

(a) Non-Competitive Assay Formats

Immunoassays for detecting mesothelin may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of captured analyte (in this case mesothelin) is directly measured. In one preferred "sandwich" assay, for example, the capture agent (e.g., anti-mesothelin antibodies) are bound directly to a solid substrate where they are immobilized. These immobilized antibodies then capture mesothelin present in the test sample. The mesothelin thus immobilized is then bound by a labeling agent, such as a second human mesothelin antibody bearing a label. Alternatively, the second mesothelin antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived.

Sandwich assays for mesothelin may be constructed. As described above, the immobilized anti-mesothelin specifically binds to mesothelin present in the sample. The labeled anti-mesothelin then binds to the already bound mesothelin. Free labeled anti-mesothelin is washed away and the remaining bound labeled anti-mesothelin is detected (e.g., using a gamma detector where the label is radioactive).

(b) Competitive Assay Formats

In competitive assays, the amount of analyte (e.g., mesothelin) present in the sample is measured indirectly by measuring the amount of an added (exogenous) analyte displaced

(or competed away) from a capture agent (e.g., anti-mesothelin antibody) by the analyte present in the sample. In one competitive assay, a known amount of analyte is added to the sample and the sample is contacted with a capture agent, in this case an antibody that specifically binds the analyte. The amount of analyte bound to the antibody is inversely proportional to the concentration of analyte present in the sample.

In a particularly preferred embodiment, the capture agent is immobilized on a solid substrate. The amount of mesothelin bound to the capture agent is determined either by measuring the amount of mesothelin present in an mesothelin/antibody complex, or alternatively by measuring the amount of remaining uncomplexed mesothelin. The amount of mesothelin may be detected by providing a labeled mesothelin.

A hapten inhibition assay is another preferred competitive assay. In this assay, a known analyte, in this case mesothelin, is immobilized on a solid substrate. A known amount of anti-mesothelin antibody is added to the sample, and the sample is then contacted with the immobilized mesothelin. In this case, the amount of anti-mesothelin antibody bound to the immobilized mesothelin is proportional to the amount of mesothelin present in the sample. Again the amount of immobilized antibody is detected by detecting either the immobilized fraction of antibody or the fraction of the antibody that remains in solution. Detection may be direct where the antibody is labeled, or indirect by the subsequent addition of a labeled moiety that specifically binds to the antibody as described above.

(c) Generation of pooled antisera
for use in immunoassays.

A mesothelin protein that specifically binds to or that is specifically immunoreactive with an antibody generated against a defined immunogen, such as an immunogen consisting of the amino acid sequence of SEQ. I.D. NO. 2, is determined in an immunoassay. The immunoassay uses a polyclonal antiserum which was raised to the protein of SEQ. I.D. NO. 2 (the immunogenic polypeptide).

In order to produce antisera for use in an immunoassay, the polypeptide of SEQ. I.D. NO. 2 is isolated as described herein. For example, recombinant protein can be produced in a mammalian or other eukaryotic cell line. An inbred strain of mice is immunized with the protein of SEQ. I.D. NO. 2 using a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane, *supra*). Alternatively, a synthetic polypeptide derived from the sequences disclosed herein and conjugated to a carrier protein is used as an immunogen. Polyclonal sera are collected and titered against the immunogenic polypeptide in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against proteins of interest, using a competitive binding immunoassay such as the one described in Harlow and Lane, *supra*, at pages 570-573.

Immunoassays in the competitive binding format are used for crossreactivity determinations. For example, the immunogenic polypeptide is immobilized to a solid support. Proteins added to the assay compete with the binding of the antisera to the immobilized antigen. The ability of the above proteins to compete with the binding of the antisera to the immobilized protein is compared to the immunogenic polypeptide. The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% crossreactivity with the protein of interest are combined and pooled. The cross-reacting antibodies are then removed from the pooled antisera by immunoadsorption. The immunoadsorbed and pooled antisera are then used in a competitive binding immunoassay as described herein to compare a second "target" polypeptide to the immunogenic polypeptide. In order to make this comparison, the two polypeptides are each assayed at a wide range of concentrations and the amount of each polypeptide required to inhibit 50% of the binding of the antisera to the immobilized protein is determined using standard techniques. If the

amount of the target polypeptide required is less than twice the amount of the immunogenic polypeptide that is required, then the target polypeptide is said to specifically bind to an antibody generated to the immunogenic protein. As a final determination of specificity, the pooled antisera is fully immunoadsorbed with the immunogenic polypeptide until no binding to the polypeptide used in the immunoadsorption is detectable. The fully immunoadsorbed antisera is then tested for reactivity with the test polypeptide. If no reactivity is observed, then the test polypeptide is specifically bound by the antisera elicited by the immunogenic protein.

D. Other Assay Formats

Western blot analysis can also be used to detect and quantify the presence of mesothelin in the sample. The technique generally comprises separating sample proteins by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support, (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with the antibodies that specifically bind mesothelin. The anti-mesothelin antibodies specifically bind to mesothelin on the solid support. These antibodies may be directly labeled or alternatively may be subsequently detected using labeled antibodies (e.g., labeled sheep anti-mouse antibodies where the antibody to mesothelin is a murine antibody) that specifically bind to the anti-mesothelin.

Other assay formats include liposome immunoassays (LIAs), which use liposomes designed to bind specific molecules (e.g., antibodies) and release encapsulated reagents or markers. The released chemicals are then detected according to standard techniques (see, Monroe et al., (1986) *Amer. Clin. Prod. Rev.* 5:34-41), which is incorporated herein by reference.

E. Labels

The labeling agent for the applications described herein can be, e.g., a monoclonal antibody, a polyclonal antibody, a mesothelin binding protein or complex such as those described herein, or a polymer such as an affinity

matrix, carbohydrate or lipid. Detection may proceed by any known method, such as immunoblotting, western analysis, gel-mobility shift assays, fluorescent *in situ* hybridization analysis (FISH), tracking of radioactive or bioluminescent markers, nuclear magnetic resonance, electron paramagnetic resonance, stopped-flow spectroscopy, column chromatography, capillary electrophoresis, or other methods which track a molecule based upon an alteration in size and/or charge. The particular label or detectable group used in the assay is not a critical aspect of the invention. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and, in general, any label useful in such methods can be applied to the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include magnetic beads (e.g. DynabeadsTM), fluorescent dyes (e.g., fluorescein isothiocyanate, texas red, rhodamine, and the like), radiolabels (e.g., ³H, ¹²⁵I, ³⁵S, ¹⁴C, or ³²P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g. polystyrene, polypropylene, latex, etc.) beads.

The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on the sensitivity required, ease of conjugation of the compound, stability requirements, available instrumentation, and disposal provisions.

Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (e.g., biotin) is covalently bound to the molecule. The ligand then binds to an anti-ligand (e.g., streptavidin) molecule which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-

ligands can be used. Where a ligand has a natural anti-ligand, for example, biotin, thyroxine, and cortisol, it can be used in conjunction with the labeled, naturally occurring anti-ligands. Alternatively, any haptenic or antigenic compound can be used in combination with an antibody.

The molecules can also be conjugated directly to signal generating compounds, e.g., by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescent compounds include luciferin, and 2,3-dihydrophthalazinediones, e.g., luminol. For a review of various labelling or signal producing systems which may be used, see, U.S. Patent No. 4,391,904, which is incorporated herein by reference.

Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence, e.g., by microscopy, visual inspection, via photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing appropriate substrates for the enzyme and detecting the resulting reaction product. Finally, simple colorimetric labels may be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the

components need be labeled and the presence of the target antibody is detected by simple visual inspection.

F. Substrates

As mentioned above, depending upon the assay, various components, including the antigen, target antibody, or anti-human antibody, may be bound to a solid surface. Many methods for immobilizing biomolecules to a variety of solid surfaces are known in the art. For instance, the solid surface may be a membrane (e.g., nitrocellulose), a microtiter dish (e.g., PVC, polypropylene, or polystyrene), a test tube (glass or plastic), a dipstick (e.g. glass, PVC, polypropylene, polystyrene, latex, and the like), a microcentrifuge tube, or a glass, silica, plastic, metallic or polymer bead. The desired component may be covalently bound, or noncovalently attached through nonspecific bonding.

A wide variety of organic and inorganic polymers, both natural and synthetic may be employed as the material for the solid surface. Illustrative polymers include polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and the like. Other materials which may be employed, include paper, glasses, ceramics, metals, metalloids, semiconductive materials, cements or the like. In addition, substances that form gels, such as proteins (e.g., gelatins), lipopolysaccharides, silicates, agarose and polyacrylamides can be used. Polymers which form several aqueous phases, such as dextrans, polyalkylene glycols or surfactants, such as phospholipids, long chain (12-24 carbon atoms) alkyl ammonium salts and the like are also suitable. Where the solid surface is porous, various pore sizes may be employed depending upon the nature of the system.

In preparing the surface, a plurality of different materials may be employed, e.g., as laminates, to obtain various properties. For example, protein coatings, such as gelatin can be used to avoid non-specific binding, simplify covalent conjugation, enhance signal detection or the like.

If covalent bonding between a compound and the surface is desired, the surface will usually be polyfunctional or be capable of being polyfunctionalized. Functional groups which may be present on the surface and used for linking can include carboxylic acids, aldehydes, amino groups, cyano groups, ethylenic groups, hydroxyl groups, mercapto groups and the like. The manner of linking a wide variety of compounds to various surfaces is well known and is amply illustrated in the literature. See, for example, *Immobilized Enzymes*, Ichiro Chibata, Halsted Press, New York, 1978, and Cuatrecasas, J. *Biol. Chem.* 245 3059 (1970) which are incorporated herein by reference.

In addition to covalent bonding, various methods for noncovalently binding an assay component can be used. Noncovalent binding is typically nonspecific absorption of a compound to the surface. Typically, the surface is blocked with a second compound to prevent nonspecific binding of labeled assay components. Alternatively, the surface is designed such that it nonspecifically binds one component but does not significantly bind another. For example, a surface bearing a lectin such as Concanavalin A will bind a carbohydrate containing compound but not a labeled protein that lacks glycosylation. Various solid surfaces for use in noncovalent attachment of assay components are reviewed in U.S. Patent Nos. 4,447,576 and 4,254,082, which are incorporated herein by reference.

II. Targeting Effector Molecules to Mesothelin

This invention also provides for compositions and methods for detecting the presence or absence of tumor cells bearing mesothelin. These methods involve providing a chimeric molecule comprising an effector molecule, that is a detectable label attached to a targeting molecule that specifically binds mesothelin. The mesothelin targeting moiety specifically binds the chimeric molecule to tumor cells which are then marked by their association with the detectable label. Subsequent detection of the cell-associated label indicates the presence of a tumor cell.

In yet another embodiment, the effector molecule may be another specific binding moiety such as an antibody, a growth factor, or a ligand. The chimeric molecule will then act as a highly specific bifunctional linker. This linker may act to bind and enhance the interaction between cells or cellular components to which the fusion protein binds. Thus, for example, where the "targeting" component of the chimeric molecule comprises a polypeptide that specifically binds to mesothelin and the "effector" component is an antibody or antibody fragment (e.g. an Fv fragment of an antibody), the targeting component specifically binds cancer cells, while the effector component inhibits cell growth or may act to enhance and direct an immune response toward target cancer cells.

In still yet another embodiment the effector molecule may be a pharmacological agent (e.g. a drug) or a vehicle containing a pharmacological agent. Thus, the moiety that specifically binds to mesothelin may be conjugated to a drug such as vinblastine, doxorubicin, genistein (a tyrosine kinase inhibitor), an antisense molecule, and other pharmacological agents known to those of skill in the art, thereby specifically targeting the pharmacological agent to tumor cells.

Alternatively, the targeting molecule may be bound to a vehicle containing the therapeutic composition. Such vehicles include, but are not limited to liposomes, micelles, various synthetic beads, and the like.

One of skill in the art will appreciate that the chimeric molecules of the present invention may include multiple targeting moieties bound to a single effector or conversely, multiple effector molecules bound to a single targeting moiety. In still other embodiments, the chimeric molecules may include both multiple targeting moieties and multiple effector molecules. Thus, for example, this invention provides for "dual targeted" cytotoxic chimeric molecules in which targeting molecule that specifically binds to mesothelin is attached to a cytotoxic molecule and another molecule (e.g. an antibody, or another ligand) is attached to the other terminus of the toxin. Such a dual-targeted

cytotoxin might comprise a growth factor substituted for domain Ia, for example, at the amino terminus of a PE and anti-TAC(Fv) inserted in domain III, between amino acid 604 and 609. Other antibodies may also be suitable.

5 A. *The Targeting Molecule*

In a preferred embodiment, the targeting molecule is a molecule that specifically binds to mesothelin. A variety of immunoassay formats may be used to select appropriate antibodies and are discussed above.

10 B. *The Effector Molecule*

As described above, the effector molecule component of the chimeric molecules of this invention may be any molecule whose activity it is desired to deliver to cells that express mesothelin. Particularly preferred effector molecules include cytotoxins such as PE or DT, radionuclides, ligands such as growth factors, antibodies, detectable labels such as fluorescent or radioactive labels, and therapeutic compositions such as liposomes and various drugs.

1. *Cytotoxins*

Particularly preferred cytotoxins include *Pseudomonas* exotoxins, *Diphtheria* toxins, ricin, and abrin. *Pseudomonas* exotoxin and *Diphtheria* toxin are most preferred.

(a) *Pseudomonas* exotoxin (PE)

Pseudomonas exotoxin A (PE) is an extremely active monomeric protein (molecular weight 66 kD), secreted by *Pseudomonas aeruginosa*, which inhibits protein synthesis in eukaryotic cells through the inactivation of elongation factor 2 (EF-2) by catalyzing its ADP-ribosylation (catalyzing the transfer of the ADP ribosyl moiety of oxidized NAD onto EF-2).

The toxin contains three structural domains that act in concert to cause cytotoxicity. Domain Ia (amino acids 1-252) mediates cell binding. Domain II (amino acids 253-364) is responsible for translocation into the cytosol and domain III (amino acids 400-613) mediates ADP ribosylation of elongation factor 2, which inactivates the protein and causes cell death. The function of domain Ib (amino acids 365-399) remains undefined, although a large part of it, amino acids 365-380, can be deleted without loss of cytotoxicity. See

Siegall et al., *J. Biol. Chem.* 264: 14256-14261 (1989), incorporated by reference herein.

Where the targeting molecule is fused to PE, a preferred PE molecule is one in which domain Ia (amino acids 1 through 252) is deleted and amino acids 365 to 380 have been deleted from domain Ib. However all of domain Ib and a portion of domain II (amino acids 350 to 394) can be deleted, particularly if the deleted sequences are replaced with a linking peptide such as GGGGS [SEQ. I.D. NO. 3].

In addition, the PE molecules can be further modified using site-directed mutagenesis or other techniques known in the art, to alter the molecule for a particular desired application. Means to alter the PE molecule in a manner that does not substantially affect the functional advantages provided by the PE molecules described here can also be used and such resulting molecules are intended to be covered herein.

For maximum cytotoxic properties of a preferred PE molecule, several modifications to the molecule are recommended. An appropriate carboxyl terminal sequence to the recombinant molecule is preferred to translocate the molecule into the cytosol of target cells. Amino acid sequences which have been found to be effective include, REDLK [SEQ. I.D. NO. 4] (as in native PE), REDL [SEQ. I.D. NO. 5], RDEL [SEQ. I.D. NO. 6], or KDEL [SEQ. I.D. NO. 7], repeats of those, or other sequences that function to maintain or recycle proteins into the endoplasmic reticulum, referred to here as "endoplasmic retention sequences". See, for example, Chaudhary et al, *Proc. Natl. Acad. Sci. USA* 87:308-312 and Seetharam et al, *J. Biol. Chem.* 266: 17376-17381 (1991) and commonly assigned, USSN 07/459,635 filed January 2, 1990, all of which are incorporated by reference herein.

Deletions of amino acids 365-380 of domain Ib can be made without loss of activity. Further, amino acids 1-279 may be deleted so that the toxin begins with a methionine followed by glycine at position 280. A serine may be placed at position 289 to prevent formation of improper disulfide bonds

is beneficial. The targeting molecule may be inserted in replacement for domain Ia.

Preferred forms of PE contain amino acids 253-364 and 381-608, and are followed by the native sequences REDLK [SEQ. I.D. NO. 4] or the mutant sequences KDEL [SEQ. I.D. NO. 7] or RDEL [SEQ. I.D. NO. 6]. Lysines at positions 590 and 606 may or may not be mutated to glutamine.

The targeting molecule may also be inserted at a point within domain III of the PE molecule. Most preferably the targeting molecule is fused between about amino acid positions 607 and 609 of the PE molecule. This means that the targeting molecule is inserted after about amino acid 607 of the molecule and an appropriate carboxyl end of PE is recreated by placing amino acids about 604-613 of PE after the targeting molecule. Thus, the targeting molecule is inserted within the recombinant PE molecule after about amino acid 607 and is followed by amino acids 604-613 of domain III. The targeting molecule may also be inserted into domain Ib to replace sequences not necessary for toxicity. Debinski, et al. *Mol. Cell. Biol.*, 11: 1751-1753 (1991).

Methods of cloning genes encoding PE fused to various ligands are well known to those of skill in the art. See, for example, Siegall et al., *FASEB J.*, 3: 2647-2652 (1989); Chaudhary et al. *Proc. Natl. Acad. Sci. USA*, 84: 4538-4542 (1987), which are incorporated herein by reference.

Those skilled in the art will realize that additional modifications, deletions, insertions and the like may be made to the chimeric molecules of the present invention or to the nucleic acid sequences encoding mesothelin-directed chimeric molecules. All such constructions may be made by methods of genetic engineering well known to those skilled in the art (see, generally, Sambrook et al., *supra*) and may produce proteins that have differing properties of affinity, specificity, stability and toxicity that make them particularly suitable for various clinical or biological applications.

(b) Diphtheria Toxin (DT)

Like PE, diphtheria toxin (DT) kills cells by ADP-ribosylating elongation factor 2 thereby inhibiting protein synthesis. Diphtheria toxin, however, is divided into two chains, A and B, linked by a disulfide bridge. In contrast to PE, chain B of DT, which is on the carboxyl end, is responsible for receptor binding and chain A, which is present on the amino end, contains the enzymatic activity (Uchida et al., *Science*, 175: 901-903 (1972); Uchida et al. *J. Biol. Chem.*, 248: 3838-3844 (1973)).

In a preferred embodiment, the targeting molecule-Diphtheria toxin fusion proteins of this invention have the native receptor-binding domain removed by truncation of the Diphtheria toxin B chain. Particularly preferred is DT388, a DT in which the carboxyl terminal sequence beginning at residue 389 is removed. Chaudhary, et al., *Bioch. Biophys. Res. Comm.*, 180: 545-551 (1991).

Like the PE chimeric cytotoxins, the DT molecules may be chemically conjugated to a mesothelin targeting molecule, but, in a preferred embodiment, the targeting molecule will be fused to the Diphtheria toxin by recombinant means. The genes encoding protein chains may be cloned in cDNA or in genomic form by any cloning procedure known to those skilled in the art. Methods of cloning genes encoding DT fused to various ligands are also well known to those of skill in the art. See, for example, Williams et al. *J. Biol. Chem.* 265: 11885-11889 (1990) and copending patent application (USSN 07/620,939) which describe the expression of a number of growth-factor-DT fusion proteins.

The term "Diphtheria toxin" (DT) as used herein refers to full length native DT or to a DT that has been modified. Modifications typically include removal of the targeting domain in the B chain and, more specifically, involve truncations of the carboxyl region of the B chain.

Detectable labels suitable for use as the effector molecule component of the chimeric molecules of this invention include any composition detectable by spectroscopic,

photochemical, biochemical, immunochemical, electrical, optical or chemical means all as described above.

C. *Attachment of the Targeting Molecule to the Effector Molecule*

One of skill will appreciate that the targeting molecule and effector molecules may be joined together in any order. Thus, where the targeting molecule is a polypeptide, the effector molecule may be joined to either the amino or carboxy termini of the targeting molecule. The targeting molecule may also be joined to an internal region of the effector molecule, or conversely, the effector molecule may be joined to an internal location of the targeting molecule, as long as the attachment does not interfere with the respective activities of the molecules.

The targeting molecule and the effector molecule may be attached by any of a number of means well known to those of skill in the art. Typically the effector molecule is conjugated, either directly or through a linker (spacer), to the targeting molecule. However, where both the effector molecule and the targeting molecule are polypeptides it is preferable to recombinantly express the chimeric molecule as a single-chain fusion protein.

D. *Conjugation of the Effector Molecule to the Targeting Molecule*

In one embodiment, the targeting molecule is chemically conjugated to the effector molecule (e.g. a cytotoxin, a label, a ligand, or a drug or liposome). Means of chemically conjugating molecules are well known to those of skill.

The procedure for attaching an agent to an antibody or other polypeptide targeting molecule will vary according to the chemical structure of the agent. Polypeptides typically contain variety of functional groups; e.g., carboxylic acid (COOH) or free amine (-NH₂) groups, which are available for reaction with a suitable functional group on an effector molecule to bind the effector thereto.

Alternatively, the targeting molecule and/or effector molecule may be derivatized to expose or attach additional reactive functional groups. The derivatization may

involve attachment of any of a number of linker molecules such as those available from Pierce Chemical Company, Rockford Illinois.

5 A "linker", as used herein, is a molecule that is used to join the targeting molecule to the effector molecule. The linker is capable of forming covalent bonds to both the targeting molecule and to the effector molecule. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where the targeting molecule and the effector molecule are polypeptides, the linkers may be joined to the constituent amino acids through their side groups (e.g., through a disulfide linkage to cysteine). However, in a preferred embodiment, the linkers will be joined to the alpha carbon amino and carboxyl groups of the terminal amino acids.

10 A bifunctional linker having one functional group reactive with a group on a particular agent, and another group reactive with an antibody, may be used to form the desired immunoconjugate. Alternatively, derivatization may involve chemical treatment of the targeting molecule, e.g., glycol cleavage of the sugar moiety of a the glycoprotein antibody with periodate to generate free aldehyde groups. The free aldehyde groups on the antibody may be reacted with free amine or hydrazine groups on an agent to bind the agent thereto. (See U.S. Patent No. 4,671,958). Procedures for generation of free sulfhydryl groups on polypeptide, such as antibodies or antibody fragments, are also known (See U.S. Pat. No. 4,659,839).

25 Many procedures and linker molecules for attachment of various compounds including radionuclide metal chelates, toxins and drugs to proteins such as antibodies are known. See, for example, European Patent Application No. 188,256; U.S. Patent Nos. 4,671,958, 4,659,839, 4,414,148, 4,699,784; 35 4,680,338; 4,569,789; and 4,589,071; and Borlinghaus et al. Cancer Res. 47: 4071-4075 (1987) which are incorporated herein by reference. In particular, production of various immunotoxins is well-known within the art and can be found,

for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al., *Monoclonal Antibodies in Clinical Medicine*, Academic Press, pp. 168-190 (1982), Waldmann, *Science*, 252: 1657 (1991), U.S. Patent Nos. 4,545,985 and 4,894,443 which are incorporated herein by reference.

In some circumstances, it is desirable to free the effector molecule from the targeting molecule when the chimeric molecule has reached its target site. Therefore, chimeric conjugates comprising linkages which are cleavable in the vicinity of the target site may be used when the effector is to be released at the target site. Cleaving of the linkage to release the agent from the antibody may be prompted by enzymatic activity or conditions to which the immunoconjugate is subjected either inside the target cell or in the vicinity of the target site. When the target site is a tumor, a linker which is cleavable under conditions present at the tumor site (e.g. when exposed to tumor-associated enzymes or acidic pH) may be used.

A number of different cleavable linkers are known to those of skill in the art. See U.S. Pat. Nos. 4,618,492; 4,542,225, and 4,625,014. The mechanisms for release of an agent from these linker groups include, for example, irradiation of a photolabile bond and acid-catalyzed hydrolysis. U.S. Pat. No. 4,671,958, for example, includes a description of immunoconjugates comprising linkers which are cleaved at the target site *in vivo* by the proteolytic enzymes of the patient's complement system. In view of the large number of methods that have been reported for attaching a variety of radiodiagnostic compounds, radiotherapeutic compounds, drugs, toxins, and other agents to antibodies one skilled in the art will be able to determine a suitable method for attaching a given agent to an antibody or other polypeptide.

E. Production of Fusion Proteins

Where the targeting molecule and/or the effector molecule is relatively short (i.e., less than about 50 amino acids) they may be synthesized using standard chemical peptide

synthesis techniques. Where both molecules are relatively short the chimeric molecule may be synthesized as a single contiguous polypeptide. Alternatively the targeting molecule and the effector molecule may be synthesized separately and then fused by condensation of the amino terminus of one molecule with the carboxyl terminus of the other molecule thereby forming a peptide bond. Alternatively, the targeting and effector molecules may each be condensed with one end of a peptide spacer molecule thereby forming a contiguous fusion protein.

Solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence is the preferred method for the chemical synthesis of the polypeptides of this invention. Techniques for solid phase synthesis are described by Barany and Merrifield, *Solid-Phase Peptide Synthesis*; pp. 3-284 in *The Peptides: Analysis, Synthesis, Biology. Vol. 2: Special Methods in Peptide Synthesis, Part A.*, Merrifield, et al. *J. Am. Chem. Soc.*, 85: 2149-2156 (1963), and Stewart et al., *Solid Phase Peptide Synthesis*, 2nd ed. Pierce Chem. Co., Rockford, Ill. (1984) which are incorporated herein by reference.

In a preferred embodiment, the chimeric fusion proteins of the present invention are synthesized using recombinant DNA methodology. Generally this involves creating a DNA sequence that encodes the fusion protein, placing the DNA in an expression cassette under the control of a particular promoter, expressing the protein in a host, isolating the expressed protein and, if required, renaturing the protein.

DNA encoding the fusion proteins of this invention may be prepared by any suitable method, including, for example, cloning and restriction of appropriate sequences or direct chemical synthesis by methods such as the phosphotriester method of Narang et al. *Meth. Enzymol.* 68: 90-99 (1979); the phosphodiester method of Brown et al., *Meth. Enzymol.* 68: 109-151 (1979); the diethylphosphoramidite method

of Beaucage et al., Tetra. Lett., 22: 1859-1862 (1981); and the solid support method of U.S. Patent No. 4,458,066, all incorporated by reference herein.

Chemical synthesis produces a single stranded oligonucleotide. This may be converted into double stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill would recognize that while chemical synthesis of DNA is limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

Alternatively, subsequences may be cloned and the appropriate subsequences cleaved using appropriate restriction enzymes. The fragments may then be ligated to produce the desired DNA sequence.

While the two molecules are preferably essentially directly joined together, one of skill will appreciate that the molecules may be separated by a peptide spacer consisting of one or more amino acids. Generally the spacer will have no specific biological activity other than to join the proteins or to preserve some minimum distance or other spatial relationship between them. However, the constituent amino acids of the spacer may be selected to influence some property of the molecule such as the folding, net charge, or hydrophobicity.

The nucleic acid sequences encoding the fusion proteins may be expressed in a variety of host cells, including *E. coli*, other bacterial hosts, yeast, and various higher eukaryotic cells such as the COS, CHO and HeLa cell lines and myeloma cell lines. The recombinant protein gene will be operably linked to appropriate expression control sequences for each host. For *E. coli* this includes a promoter such as the T7, trp, or lambda promoters, a ribosome binding site and preferably a transcription termination signal. For eukaryotic cells, the control sequences will include a promoter and preferably an enhancer derived from immunoglobulin genes, SV40, cytomegalovirus, etc., and a

polyadenylation sequence, and may include splice donor and acceptor sequences.

The plasmids and vectors of the invention can be transferred into the chosen host cell by well-known methods such as calcium chloride transformation for *E. coli* and calcium phosphate treatment or electroporation for mammalian cells. Cells transformed by the plasmids can be selected by resistance to antibiotics conferred by genes contained on the plasmids, such as the amp, gpt, neo and hyg genes.

Once expressed, the recombinant fusion proteins can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see, generally, R. Scopes, *Protein Purification*, Springer-Verlag, N.Y. (1982), Deutscher, *Methods in Enzymology* Vol. 182: *Guide to Protein Purification.*, Academic Press, Inc. N.Y. (1990)). Substantially pure compositions of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity are most preferred for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically.

III. *Administration to Patients of Targeting Agents to Mesothelin*

Therapeutic agents of the present invention, such as antibodies to mesothelin or such as antibodies or other targeting molecules attached to an effector molecule are administered in any suitable manner, preferably with pharmaceutically acceptable carriers. One skilled in the art will appreciate that suitable methods of administering such compounds in the context of the present invention to a patient are available, and, although more than one route can be used to administer a particular compound, a particular route can often provide a more immediate and more effective reaction than another route. It should be recognized that the administration of peptides are well-known for a variety of diseases, and one of skill is able to extrapolate the

information available for use of peptides to treat these other diseases to mesothelin peptides.

Pharmaceutically acceptable carriers are also well known to those who are skilled in the art. The optimal choice of carrier will be determined in part by the particular compound, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of the pharmaceutical compositions of the present invention.

Antibodies may be formulated into an injectable preparation. Parenteral formulations are known and are suitable for use in the invention, preferably for i.m. or i.v. administration. The formulations containing therapeutically effective amounts of antibodies or immunotoxins are either sterile liquid solutions, liquid suspensions or lyophilized versions and optionally contain stabilizers or excipients. Lyophilized compositions are reconstituted with suitable diluents, e.g., water for injection, saline, 0.3% glycine and the like, at a level of about from .01 mg/kg of host body weight to 10 mg/kg where appropriate. Typically, the pharmaceutical compositions containing the antibodies or immunotoxins will be administered in a therapeutically effective dose in a range of from about .01 mg/kg to about 5 mg/kg of the treated mammal. A preferred therapeutically effective dose of the pharmaceutical composition containing antibody or immunotoxin will be in a range of from about 0.01 mg/kg to about 0.5 mg/kg body weight of the treated mammal administered over several days to two weeks by daily intravenous infusion, each given over a one hour period, in a sequential patient dose-escalation regimen.

Antibody may be administered systemically by injection i.m., subcutaneously, intrathecally or intraperitoneally or into vascular spaces, particularly into the peritoneal cavity or thoracic cavity, e.g., injection at a dosage of greater than about 1 μ g/cc fluid/day. A permanent intrathecal catheter would be a convenient means to administer therapeutic antibodies. The dose will be dependent upon the properties of the antibody or immunotoxin employed, e.g., its

activity and biological half-life, the concentration of antibody in the formulation, the site and rate of dosage, the clinical tolerance of the patient involved, the disease afflicting the patient and the like as is well within the skill of the physician.

The antibody of the present invention may be administered in solution. The pH of the solution should be in the range of pH 5 to 9.5, preferably pH 6.5 to 7.5. The antibody or derivatives thereof should be in a solution having a suitable pharmaceutically acceptable buffer such as phosphate, tris (hydroxymethyl) aminomethane-HCl or citrate and the like. Buffer concentrations should be in the range of 1 to 100 mM. The solution of antibody may also contain a salt, such as sodium chloride or potassium chloride in a concentration of 50 to 150 mM. An effective amount of a stabilizing agent such as an albumin, a globulin, a gelatin, a protamine or a salt of protamine may also be included and may be added to a solution containing antibody or immunotoxin or to the composition from which the solution is prepared. Antibody or immunotoxin may also be administered via microspheres, liposomes or other microparticulate delivery systems placed in certain tissues including blood.

Dosages

In therapeutic applications, the dosages of compounds used in accordance with the invention vary depending on the class of compound and the condition being treated. The age, weight, and clinical condition of the recipient patient; and the experience and judgment of the clinician or practitioner administering the therapy are among the factors affecting the selected dosage. For example, the dosage of an immunoglobulin can range from about 0.1 milligram per kilogram of body weight per day to about 10 mg/kg per day for polyclonal antibodies and about 5% to about 20% of that amount for monoclonal antibodies. In such a case, the immunoglobulin can be administered once daily as an intravenous infusion. Preferably, the dosage is repeated daily until either a therapeutic result is achieved or until side effects warrant discontinuation of therapy. Generally, the dose should be

sufficient to treat or ameliorate symptoms or signs of the disease without producing unacceptable toxicity to the patient.

A therapeutically effective amount of the compound is that which provides either subjective relief of a symptom(s) or an objectively identifiable improvement, such as inhibition of tumor cell growth, as noted by the clinician or other qualified observer. The dosing range varies with the compound used, the route of administration and the potency of the particular compound.

IV. *Gene Therapy and Inhibitory Nucleic Acid Therapeutics*

Using the nucleotide sequence information of this invention, one skilled in the art can formulate strategies and methods to isolate the mesothelin gene, describe the gene structure for function, and may also discover specific promoters for known or unknown transcriptional factors which may be of further value in the genetic intervention of mesothelioma and ovarian cancers. Analytical DNA sequencing of normal mesothelin in mesothelial cells may lead to a discovery of mutation(s) of the gene in mesothelioma and ovarian cancers.

Mesothelin manifests an adhesive property which can be attributed to implantation of the mesothelioma and ovarian cancers. By introducing antisense DNA or blocking the transcription of mesothelin gene, novel gene therapy regimens can be set up according to current strategies of gene therapy.

Inhibitory nucleic acid therapeutics which can block the expression or activity of the mesothelin gene will be useful in slowing or inhibiting the growth of mesotheliomas or ovarian tumors or other abnormal cells which are associated with mesothelin. Inhibitory nucleic acids may be single-stranded nucleic acids, which can specifically bind to a complementary nucleic acid sequence. By binding to the appropriate target sequence, an RNA-RNA, a DNA-DNA, or RNA-DNA duplex or triplex is formed. These nucleic acids are often termed "antisense" because they are usually complementary to the sense or coding strand of the gene, although recently

approaches for use of "sense" nucleic acids have also been developed. The term "inhibitory nucleic acids" as used herein, refers to both "sense" and "antisense" nucleic acids.

By binding to the target nucleic acid, the inhibitory nucleic acid can inhibit the function of the target nucleic acid. This could, for example, be a result of blocking DNA transcription, processing or poly(A) addition to mRNA, DNA replication, translation, or promoting inhibitory mechanisms of the cells, such as promoting RNA degradation. Inhibitory nucleic acid methods therefore encompass a number of different approaches to altering expression of, for example, a mesothelin gene. These different types of inhibitory nucleic acid technology are described in Helene, C. and Toulme, J., 1990, *Biochim. Biophys. Acta.* 1049:99-125, which is hereby incorporated by reference and is referred to hereinafter as "Helene and Toulme."

In brief, inhibitory nucleic acid therapy approaches can be classified into those that target DNA sequences, those that target RNA sequences (including pre-mRNA and mRNA), those that target proteins (sense strand approaches), and those that cause cleavage or chemical modification of the target nucleic acids.

Approaches targeting DNA fall into several categories. Nucleic acids can be designed to bind to the duplex DNA to form a triple helical or "triplex" structure. Alternatively, inhibitory nucleic acids are designed to bind to regions of single stranded DNA resulting from the opening of the duplex DNA during replication or transcription. See Helene and Toulme.

More commonly, inhibitory nucleic acids are designed to bind to mRNA or mRNA precursors. Inhibitory nucleic acids are used to prevent maturation of pre-mRNA. Inhibitory nucleic acids may be designed to interfere with RNA processing, splicing or translation.

The inhibitory nucleic acids can be targeted to mRNA. In this approach, the inhibitory nucleic acids are designed to specifically block translation of the encoded protein. Using this approach, the inhibitory nucleic acid can

be used to selectively suppress certain cellular functions by inhibition of translation of mRNA encoding critical proteins. For example, an inhibitory nucleic acid complementary to regions of c-myc mRNA inhibits c-myc protein expression in a human promyelocytic leukemia cell line, HL60, which overexpresses the c-myc proto-oncogene. See Wickstrom E.L., et al., 1988, *PNAS (USA)* 85:1028-1032 and Harel-Bellan, A., et al., 1988, *Exp. Med.* 168:2309-2318. As described in Helene and Toulme, inhibitory nucleic acids targeting mRNA have been shown to work by several different mechanisms to inhibit translation of the encoded protein(s).

The inhibitory nucleic acids introduced into the cell can also encompass the "sense" strand of the gene or mRNA to trap or compete for the enzymes or binding proteins involved in mRNA translation. See Helene and Toulme.

Lastly, the inhibitory nucleic acids can be used to induce chemical inactivation or cleavage of the target genes or mRNA. Chemical inactivation can occur by the induction of crosslinks between the inhibitory nucleic acid and the target nucleic acid within the cell. Other chemical modifications of the target nucleic acids induced by appropriately derivatized inhibitory nucleic acids may also be used.

Cleavage, and therefore inactivation, of the target nucleic acids may be accomplished by attaching a substituent to the inhibitory nucleic acid which can be activated to induce cleavage reactions. The substituent can be one that affects either chemical, or enzymatic cleavage. Alternatively, cleavage can be induced by the use of ribozymes or catalytic RNA. In this approach, the inhibitory nucleic acids would comprise either naturally occurring RNA (ribozymes) or synthetic nucleic acids with catalytic activity.

The targeting of inhibitory nucleic acids to specific cells of the immune system by conjugation with targeting moieties binding receptors on the surface of these cells can be used for all of the above forms of inhibitory nucleic acid therapy. This invention encompasses all of the

forms of inhibitory nucleic acid therapy as described above and as described in Helene and Toulme.

This invention relates to the targeting of inhibitory nucleic acids to sequences of mesothelin for use in inhibiting or slowing the growth of tumors associated with mesothelin. A problem associated with inhibitory nucleic acid therapy is the effective delivery of the inhibitory nucleic acid to the target cell *in vivo* and the subsequent internalization of the inhibitory nucleic acid by that cell. Delivery, however, can be accomplished by linking the inhibitory nucleic acid to a targeting moiety to form a conjugate that binds to a specific receptor on the surface of the target infected cell, and which is internalized after binding. Preferably, the inhibitory nucleic acid will be delivered to the peritoneal cavity, the thoracic cavity, as well as any other location where cells bearing mesothelin are of interest.

Gene therapy can also correct genetic defects by insertion of exogenous cellular genes that encode a desired function into cells that lack that function, such that the expression of the exogenous gene a) corrects a genetic defect or b) causes the destruction of cells that are genetically defective. Methods of gene therapy are well known in the art, see, for example, Lu, M., et al. (1994), *Human Gene Therapy* 5:203; Smith, C. (1992), *J. Hematotherapy* 1:155; Cassel, A., et al. (1993), *Exp. Hematol.* 21:585 (1993); Larrick, J.W. and Burck, K.L., *GENE THERAPY: APPLICATION OF MOLECULAR BIOLOGY*, Elsevier Science Publishing Co., Inc., New York, New York (1991) and Kreigler, M. *GENE TRANSFER AND EXPRESSION: A LABORATORY MANUAL*, W.H. Freeman and Company, New York (1990), each incorporated herein by reference. One modality of gene therapy involves (a) obtaining from a patient a viable sample of primary cells of a particular cell type; (b) inserting into these primary cells a nucleic acid segment encoding a desired gene product; (c) identifying and isolating cells and cell lines that express the gene product; (d) re-introducing cells that express the gene product; (e) removing from the patient an aliquot of tissue including cells resulting from step c and

their progeny; and (f) determining the quantity of the cells resulting from step c and their progeny, in said aliquot. The introduction into cells in step (b) of a vector that encodes a sequence (for a "desired gene product") which will block mesothelin expression or activity can be useful in inhibiting or slowing the growth of tumor cells associated with mesothelin.

V. Vaccine Development

Vaccine development using mesothelin amino acid sequence.

Substances suitable for use as vaccines for the prevention of and inhibition of the growth of tumors bearing mesothelin and methods for administering them may be employed. The vaccines are directed against mesothelin. Preferably, the vaccines comprise mesothelin derived antigen.

Vaccines can be made recombinantly. Typically, a vaccine will include from about 1 to about 50 micrograms of antigen or antigenic protein or peptide. More preferably, the amount of protein is from about 15 to about 45 micrograms. Typically, the vaccine is formulated so that a dose includes about 0.5 milliliters. The vaccine may be administered by any route known in the art. Preferably, the route is intraperitoneally or parenteral.

There are a number of strategies for amplifying an antigen's effectiveness, particularly as related to the art of vaccines. For example, cyclization or circularization of a peptide can increase the peptide's antigenic and immunogenic potency. See U.S. Pat. No. 5,001,049 which is incorporated by reference herein. More conventionally, an antigen can be conjugated to a suitable carrier, usually a protein molecule. This procedure has several facets. It can allow multiple copies of an antigen, such as a peptide, to be conjugated to a single larger carrier molecule. Additionally, the carrier may possess properties which facilitate transport, binding, absorption or transfer of the antigen.

For parenteral administration, examples of suitable carriers are the tetanus toxoid, the diphtheria toxoid, serum albumin and lamprey, or keyhole limpet, hemocyanin because

they provide the resultant conjugate with minimum genetic restriction. Conjugates including these universal carriers can function as T cell clone activators in individuals having very different gene sets.

5 The conjugation between a peptide and a carrier can be accomplished using one of the methods known in the art. Specifically, the conjugation can use bifunctional cross-linkers as binding agents as detailed, for example, by Means and Feeney, "A recent review of protein modification
10 techniques," *Bioconjugate Chem.* 1:2-12 (1990).

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5 The antigen may be combined or mixed with various solutions and other compounds as is known in the art. For example, it may be administered in water, saline or buffered vehicles with or without various adjuvants or immunodiluting agents. Examples of such adjuvants or agents include aluminum hydroxide, aluminum phosphate, aluminum potassium sulfate (alum), beryllium sulfate, silica, kaolin, carbon, water-in-oil emulsions, oil-in-water emulsions, muramyl dipeptide, bacterial endotoxin, lipid X, *Corynebacterium parvum* (Propionibacterium acnes), *Bordetella pertussis*, polyribonucleotides, sodium alginate, lanolin, lysolecithin, vitamin A, saponin, liposomes, levamisole, DEAE-dextran, blocked copolymers or other synthetic adjuvants. Such adjuvants are available commercially from various sources, for
25 example, Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.) or Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Michigan). Other suitable adjuvants are Amphigen (oil-in-water), Alhydrogel (aluminum hydroxide), or a mixture of Amphigen and Alhydrogel. Only
30 aluminum is approved for human use.

35 The proportion of antigen and adjuvant can be varied over a broad range so long as both are present in effective amounts. For example, aluminum hydroxide can be present in an amount of about 0.5% of the vaccine mixture (Al_2O_3 basis). On a per-dose basis, the amount of the antigen can range from about 0.1 μg to about 100 μg protein per patient. A preferable range is from about 1 μg to about 50 μg per dose. A more preferred range is about 15 μg to about 45 μg . A

suitable dose size is about 0.5 ml. After formulation, the vaccine may be incorporated into a sterile container which is then sealed and stored at a low temperature, for example 4°C, or it may be freeze-dried. Lyophilization permits long-term storage in a stabilized form.

The treatment may consist of a single dose of vaccine or a plurality of doses over a period of time. It is preferred that the doses be given to a patient suspected of having mesothelin bearing tumor cells. The antigen of the invention can be combined with appropriate doses of compounds including influenza antigens, such as influenza type A antigens. Also, the antigen could be a component of a recombinant vaccine which could be adaptable for oral administration.

Vaccines of the invention may be combined with other vaccines for other diseases to produce multivalent vaccines. A pharmaceutically effective amount of the antigen can be employed with a pharmaceutically acceptable carrier such as a protein or diluent useful for the vaccination of mammals, particularly humans. Other vaccines may be prepared according to methods well-known to those skilled in the art.

Those of skill will readily recognize that it is only necessary to expose a mammal to appropriate epitopes in order to elicit effective immunoprotection. The epitopes are typically segments of amino acids which are a small portion of the whole protein. Using recombinant genetics, it is routine to alter a natural protein's primary structure to create derivatives embracing epitopes that are identical to or substantially the same as (immunologically equivalent to) the naturally occurring epitopes. Such derivatives may include peptide fragments, amino acid substitutions, amino acid deletions and amino acid additions within the amino acid sequence for mesothelin. For example, it is known in the protein art that certain amino acid residues can be substituted with amino acids of similar size and polarity without an undue effect upon the biological activity of the protein.

Using the mesothelin amino acid sequence information, one of skill in the art can perform epitope mapping against sera isolated from patients with ovarian cancers or mesotheliomas. Relatively strong epitopes may be identified and common epitope(s) may also be recognized. The epitope mapping against human sera can also be extended to a screening of epitope-peptides against activated human lymphocytes in order to identify potential T-cell epitopes. Theoretically, it is not likely that T-cell epitopes of mesothelin will be found in human T-cells, but mutations induced in mesothelin may create new epitopes which may be recognized by T-cells. Mutant mesothelin can easily be generated randomly using a phage display method. The resultant library is screened by human sera from patients suffering from malignant mesothelioma and ovarian cancer. Thus, suitable antigenic peptides may be identified for mesothelin-derived vaccines.

VI. *Kits.*

This invention further embraces diagnostic kits for detecting for the presence of mesothelin in tissue samples or in serum, comprising a container having a nucleic acid or an antibody or other targeting agent specific for mesothelin and instructional material for the detection of mesothelin.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

All publications and patent applications cited in this specification are herein incorporated by reference in their entirety for all purposes as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

VI. *Models for Evaluation of Therapies Directed to Mesothelin.*

The mesothelin cDNA can be transfected into established tumor cell lines where it will express the protein. The transfected cell lines can be used to grow tumors in mice or other mammals to provide a model for testing therapies directed to controlling, suppressing or regulating mesothelin expression. Transfected tumor cell lines can be transplanted into the test mammal. The mammal can then be subjected to a drug of interest and subsequent tumor cell activity can be monitored to determine whether the drug of interest has anti-tumor effects. Tumor cell lines that have been found to be particularly good candidates for this procedure include, mouse NIH 3T3 cells (tumorigenic cell lines), A431 human ovarian tumor cells and MCF-7 breast tumor cells, A2780 human ovarian tumor cells and OVCAR-3 human epidermoid carcinoma cells.

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Examples

A. Materials and Methods

1. **Cells and antibodies.** Human ovarian tumor cell line, OVCAR-3, and cell lines A431, KB, MCF-7, COS-1, WI-38 and NIH 3T3 were obtained from the American Type Culture Collections (ATCC, Rockville, Maryland). Cells were cultured either in RPMI 1640 or DMEM media (GIBCO Laboratories, Grand Island, NY), supplemented with L-glutamine (2 mM), penicillin (50 µg/ml), streptomycin (50 units/ml) and 5-10% fetal bovine serum (GIBCO). NIH 3T3 transfectants were grown in DMEM with 0.8 mg/ml of G418 (GIBCO). Cells were used when they reached 80-90% confluency after washing three times with ice-cold PBS (GIBCO). MAb K1 and antibody MOPC-21 have been described (Chang, K., et al., *Int. J. Cancer* 50, 373-381 (1992)) and were used in a concentration of 5-10 µg/ml.

2. **Isolation of the cDNA clones.** The HeLa S3 cDNA library (ClonTech, Palo Alto, CA) was screened at approximately 50,000 pfu/150 mm filter as described previously (Chang, K., and Pastan, I., *Int. J. Cancer* 57, 90-97 (1994)) using protein A-purified MAb K1 (5 µg/ml) and peroxidase-conjugated goat anti-mouse IgG (H+L) (10 µg/ml, Jackson ImmunoResearch Lab, Inc., West Grove, PA). Two positive plaques (λ6-1, λ6-2) were isolated and the phages were purified to homogeneity by three or more rounds of screening. After verification of their specificity with MAb K1 by showing they did not react with a control MOPC-21 antibody, single-plaque isolates of λ6-1 and λ6-2 were used to prepare 5 to 10 phage-plates, followed by extraction and purification of phage DNA with a lambda phage DNA kit (Qiagen, Inc., Chatsworth, CA). Phage DNA was then digested with EcoRI and the insert subcloned into the EcoRI site of a pCDNAI/Amp (Invitrogen Corporation, San Diego, CA) vector using a rapid ligation protocol (Chang, K., and Pastan, I., *Int. J. Cancer* 57, 90-97 (1994)). Plasmid DNAs were isolated using Qiagene's plasmid DNA isolation kit (Chang, K., and Pastan, I., *Int. J. Cancer* 57, 90-97 (1994)). Restriction mapping using XhoI, EcoRI, SalI, BamHI, NcoI, and DNA sequencing revealed that the

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two plasmid clones (p6-1 and p6-2) had identical 1500 base-pair inserts.

To isolate a longer clone, the insert of p6-1 was purified to make a cDNA probe (specific activity = 8.5×10^5 cpm/ml) by random priming. The HeLa S3 cDNA library was re-screened using the filter hybridization method described previously (Chang, K., and Pastan, I., *Int. J. Cancer* 57, 90-97 (1994)). 14 lambda clones were isolated and purified, and their insert sizes were assessed by digestion with EcoRI. Four large inserts were subcloned into a pcDNAI/Amp plasmid vector (p9, p13-1, p16 and p18-1). p9 contained the largest insert with a long open reading frame.

3. **DNA sequencing analysis.** Using T3 and T7 promoter primers and twenty 17 bp synthetic primers, the entire cDNA insert of p9 was sequenced using the method described by Sanger (Sanger, F., et al., *Proc. Natl. Acad. Sci. USA* 74, 5463-5467 (1977)) and an automatic cycle sequencing method.

4. **Northern blot analysis.** Total RNAs (20 μ g) from OVCAR-3, KB, MCF-7, A431 and WI38 were electrophoresed on a 1% agarose gel in MOPS buffer with 16.6% formaldehyde, and then transferred to a Nylon paper. Northern hybridization was done with a method described before (Chang, K., and Pastan, I., *Int. J. Cancer* 57, 90-97 (1994)). The blot washed and reprobed with a 32 P-labeled human β -actin cDNA as an internal control to assess the integrity and quantity of the RNA samples loaded.

5. **In Vitro transcription and translation.** TNT Coupled Reticulocyte Lysate System, canine pancreatic microsomal membrane, 2 μ g of plasmid DNAs of p9 (pcDICA1-9), pAPK1 (Chang, K., and Pastan, I., *Int. J. Cancer* 57, 90-97 (1994)), to eliminate and 3 H leucine were used in an *in vitro* transcription/translation and translocation/ processing experiment according to the protocol of the manufacturer (Promega, Madison, WI, USA). Translation products were resolved on a 10% SDS-PAGE reducing gel. The proteins were fixed and the unincorporated label was removed by soaking the gel three times in 200 ml of buffer, 40% methanol and 10% acetic acid in deionized water for 30 min. The gels were then

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soaked for 30 min in 200 ml of INTENSIFY Part A and Part B (NEN Research Product, Boston, MA). After drying, the translated products were visualized by autoradiography.

6. **Expression of the cloned cDNAs in mammalian cells.** Transient transfections of COS cells were performed using pcDICA1-9 (p9) and LipofectAMINE (GIBCO) following the manufacturer's protocol (GIBCO). COS1 cells were plated a day before the experiment at 2.5×10^5 cells/60 mm dish. 24 μ l of LipofectAMINE and 76 μ l of OptiMEMI medium were mixed with 10 μ g of pcDNA1/Amp vector, or pcDICA1-9 in 100 μ l of OptiMEMI medium at room temperature for 30 min. After washing the COS-1 cells with OptiMEMI twice, 2.4 ml OptiMEMI were added to the transfection mixtures and overlaid onto COS1 cells, followed by incubation at 37°C for 5 hours. 2.6 ml of DMEM with 20% FBS were then added into each dish. 48 hours after transfection, the dishes were subjected to immunofluorescence labeling as described (Chang, K., et al., *Int. J. Cancer* 50, 373-381 (1992); Chang, K., et al., *Cancer Res.* 52, 181-186 (1992)) or other treatments. The insert from plasmid p9 (in pcDNA1/Amp) was also subcloned into a pcDNA3 (Invitrogen) vector for stable transfection. Plasmid minipreps were made using Qiagen's Miniprep Plasmid DNA Kit and orientation of the insert in individual clone was determined by restriction mapping. The resulting plasmid, pcD3CAK1-9, was then used to transfect NIH 3T3, MCF-7, A431 and OVCAR-3 cells by DNA-calcium phosphate precipitation as described (Chen, C. and Okayama, H., *Mol. Cell. Biol.* 7, 2745-2752 (1987)). After overnight exposure to the precipitate, the cells were washed with PBS three times and fed with fresh DMEM/10% FBS medium for 2 - 3 days. Geneticin G418 sulfate (0.8 mg/ml) was added and the cultures were maintained until colonies of 2-3 mm in diameter were formed. Colonies were then transferred into wells of a 96 well plate and then into a 35 mm dish when they were 80% confluent. Transfected cells were screened by immunofluorescence (Chang, K., et al., *Int. J. Cancer* 50, 373-381 (1992); Chang, K., et al., *Cancer Res.* 52, 181-186 (1992)) and positive cells were further subcloned by limited dilution as described (Chang, K., et al., *Int. J. Cancer* 50,

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373-381 (1992)). One of the NIH 3T3 transfectant clones, NIH 3T3 K20, was chosen for further study. To localize the expression of CAK1, both cell surface and intracellular immunofluorescence labeling was also performed according to methods described before (Chang, K., et al., *Cancer Res.* 52, 181-186 (1992)).

7. Treatment of the transfected cells with PI-PLC.

CAK1 cDNA transfected NIH 3T3 cells (NIH 3T3 K20) were grown in 175 mm² flasks, and when they reached 90% confluency, the cells were washed in PBS for three times. The cells were incubated with either 5 ml of 1.25 U/ml PI-PLC (from *Bacillus cereus*; Boehringer Mannheim Biochemicals) or 0.05% trypsin/0.052 mM EDTA for 30 min at 37°C and 30 min at room temperature with shaking. The supernatants were collected and after centrifugation at 1000 xg and concentrated about 10 fold using Centricon 30 (Amicon, Inc., Beverly, MA). The concentrated supernatants were used in SDS-PAGE and immunoblot analysis. The enzyme-treated cells can be recultured and the recovery of CAK1 expression can be seen after overnight culture. Treatment with PI-PLC was done in a similar manner using 35 mm diameter dishes followed by immunofluorescence labeling of the treated cells (Chang, K., et al., *Cancer Res.* 52, 181-186 (1992)).

8. Immunoblotting analysis of the transfected NIH 3T3 cells. Membrane and cytosolic fractions from transfected NIH 3T3 K20 cells (Chang, K., and Pastan, I., *Int. J. Cancer* 57, 90-97 (1994)) were subjected to 12.5% SDS-PAGE and the resolved proteins were transferred to nitrocellulose. Immunoblotting was performed as previously described (Chang, K., et al., *Int. J. Cancer* 51, 548-554 (1992); Chang, K., and Pastan, I., *Int. J. Cancer* 57, 90-97 (1994)).

B. Results

Expression cloning was used to isolate the CAK1 cDNA. We previously observed that MAb K1 reacts with OVCAR-3 and HeLa cells. Because we were unable to isolate the cDNA from an OVCAR-3 library (Chang, K., and Pastan, I., *Int. J. Cancer* 57, 90-97 (1994)), we screened a HeLa cDNA library expressed in λ gt11 as described above. A total of 1×10^6

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phages were screened and two phage clones (λ 6-1 and λ 6-2) were identified. DNA sequencing showed both phages contained the same 1.5 kb insert. The insert hybridized to mRNA from OVCAR-3 and KB cells (a HeLa subclone which also reacts with MAb K1) but not to RNA from many other cell lines indicating that the cDNA is specific for cells reacting with MAb K1. 20 μ g of total RNA from OVCAR-3 cells (lane 1), MCF-7 cells (lane 2), KB cells (a HeLa subclone, lane 3), A431 cells (lane 4) and W138 cells (lane 5) were resolved by electrophoresis transferred to nylon paper and blotted with a 32 P-labeled CAK1 probe. Hybridization with an actin probe showed that the lanes were equally loaded. The mRNA detected is 2.2 kb in size indicating that the insert isolated was not full length. The insert contained an open reading frame, a stop codon and a poly A tail but the 5' end appeared to be missing. Therefore, the phage library was rescreened with one of the inserts and 14 new phages with cDNA inserts of various sizes isolated. The largest insert (#9) was 2138 bp long and when sequenced contained an open reading frame of 1884 bp (Fig. 1). It contains a typical Kozak sequence (Kozak, M., *Nucleic Acids Res.* 5, 8125-8148 (1987)) (AXXATGG) followed by an open reading frame that encodes a 69 kD protein. The sequence was not present in various data bases examined (EMBL-GenBank). Because the CAK1 antigen was originally found to be about 40 kD in size, several experiments were carried out to determine if clone 9 encoded CAK1.

1. *In vitro* translation. Insert 9 was cloned into a pcDNAI/Amp vector to make pcDICA1-9 and used in the TNT reticulocyte system. pcDICA1-9 plasmid DNA (lanes 1 and 2), and pcDIAPK1 (lanes 3 and 4) were used in a TNT coupled reticulocyte lysate system in the presence (+) or absence (-) of pancreatic microsomal membrane (m). The products were resolved on a 10% reducing SDS-PAGE and autoradiographed. A 69 kD protein was produced. In the presence of pancreatic microsomes (lane 2), a slightly larger protein was observed indicating the protein had been inserted into microsomes and glycosylated. As a control, a cDNA encoding a 30 kD cytosolic protein that also reacts with MAb K1 (Chang, K., and Pastan,

I., *Int. J. Cancer* 57, 90-97 (1994)) was subjected to the same analysis. The size of the protein was unaffected by the presence of microsomes.

2. **Expression in cultured cells.** pcDICAk1-9 was transfected into COS cells for transient expression. pcDNA1/Amp vectors with insert 9 or without insert were transfected into COS cells. Two days later, the cells were immunocytochemically labeled with MAb K1 at 4°C (for surface labeling) or at 23°C (for intracellular labeling) and photographed (Magnification X 250). The specific labeling pattern of COS cells transfected with insert 9 using MAb K1 was observed. In nonpermeabilized cells, a typical cell surface fluorescent pattern is detected. In permeabilized cells, strong staining of the Golgi region is evident. No cytosolic staining was detected. Also, no immunoreactivity was detected in cells transfected with vector without insert or control inserts. Thus, insert 9 encodes a cell surface protein that is also present in the Golgi.

3. **Size and processing of CAK1 antigen.** To determine the size of the antigen produced by cells transfected with insert 9, NIH 3T3 cells were transfected with pcD3CAK1-9 to make stable cell lines. Stably transfected clones were produced as described above and the presence of antigen on the surface was confirmed by immunofluorescence. Then membrane and cytosolic fractions were prepared from NIH 3T3 K20 cells and from OVCAR-3 cells, subjected to SDS-PAGE and analyzed by immunoblotting with MAb K1. Approximately 100 µg of membrane fraction (lanes 1 and 3) or cytosolic fraction (lanes 2 and 4) of the transfected NIH 3T3 (pcD3CAK1) and mock control (pcD3) and membrane (lane 5) or cytosolic fraction (lane 6) of OVCAR-3 cells were electrophoresed and immunoblotted with MAb K1. As previously reported, the major reactivity in OVCAR-3 cells is with a doublet of about 40 and 43 kD that is present in membranes but not in the cytosol. In the transfectants, two bands of equal intensity were detected in the membrane fraction; one of about 40 kD and a second of about 71 kD. No signal was detected in the cytosol. These data suggest that

CAK1 is made as a large molecular weight precursor that is processed by proteolysis to an approximately 40 kD form.

4. **Nature of cell surface attachment.** To determine if CAK1 was attached to the transfectants via a PI linkage as it is in OVCAR-3 cells (Chang, K., et al., *Cancer Res.* 52, 181-186 (1992)), the NIH 3T3 transfectant cell line k20 was treated with PI-PLC for 60 min. The transfected NIH 3T3 k20 cells were treated with PI-PLC and labeled with MAb K1 as described above. The CAK1 signal was completely abolished after PI-PLC treatment. A strong cell surface labeling pattern was observed in untreated cells. Fluorescence was absent after treatment with PI-PLC. In phase contrast images before (B) and after (D) treatment, the treated cells are still attached to the dish but are slightly altered in shape. The medium from PI-PLC treated cells was concentrated, subjected to SDS-PAGE and analyzed with MAb K1. A band of about 70 kD was detected, but no lower molecular weight bands were detected.

C. Summary of Results

Thus, the above describes the molecular cloning of the CAK1 antigen which is found on mesothelium, mesotheliomas, ovarian cancers and some squamous cell carcinomas. We have designated this antigen mesothelin to reflect its presence on mesothelial cells. One unexpected feature of mesothelin is that its cDNA encodes a 69 kD protein, whereas the antigen present on OVCAR-3 cells, used to isolate MAb K1, has a molecular weight of ~40,000 Daltons. The DNA sequence and the deduced amino acid sequence of CAK1 is shown in Fig. 1. The cDNA is 2138 bp in length and contains an open reading frame of 1884 bp. The protein it encodes contains 628 amino acids with a calculated molecular weight of 69001 daltons. A homology analysis was performed with nucleotide or amino acid sequences and none was detected using EMBL-GenBank accessed by the GCG program. The protein contains four potential N-linked glycosylation sites N-X-S or N-X-T that are shown in bold letters. A typical signal sequence is not present at the amino terminus. However, a short hydrophobic segment is located 15 amino acids from the first methionine (Fig. 1).

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This sequence might function as a signal sequence for membrane insertion, because the protein is found on the cell surface and is inserted into microsomes during cell free translation. Also present is a putative proteolytic processing site, RPRFRR, beginning at amino acid 293 (Fig. 1). This site is recognized by furin, a protease important in the processing of several membrane proteins as well as in the activation of *Pseudomonas* and diphtheria toxins (Chiron, M.F., et al., *J.B.C.* 269(27):18169-18176 (1994)). The 40 kD form appears to be derived from a 69 kD precursor by several processing steps. These are summarized in Fig. 2. Initially, mesothelin is made as a 69 kD polypeptide with a hydrophobic tail which is probably removed and replaced by phosphatidylinositol (Chang, K., et al., *Cancer Res.* 52, 181-186 (1992)). After glycosylation at one or more of its four putative N-linked glycosylation sites, it is cleaved by a protease to yield the 40 kD fragment (or doublet) found on the surface of OVCAR-3 cells and a smaller (~31 kD) fragment. The latter could be released into the medium and/or further degraded. The amino terminal fragment has recently been detected in the medium of OVCAR-3 cells (our data). In transfected NIH 3T3 and MCF-7 cells, we find approximately equal amounts of 70 kD and 40 kD proteins. We originally detected the 40 kD form in OVCAR-3 and HeLa cells and did not notice a larger form. Reexamination of the OVCAR-3 and HeLa cell gels reveals a trace amount of the 70 kD precursor.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Pastan, Ira
Chang, Kai
- (ii) TITLE OF INVENTION: Mesothelin, a Differentiation Antigen
Present on Mesothelium, Mesotheliomas and Ovarian Cancers
and Methods and Kits for Targeting the Antigen
- (iii) NUMBER OF SEQUENCES: 7
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Townsend and Townsend and Crew
(B) STREET: One Market Plaza, Steuart Street Tower
(C) CITY: San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94105-1492
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Not yet assigned
(B) FILING DATE: Not yet assigned
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Weber, Ellen Lauver
(B) REGISTRATION NUMBER: 32,762
(C) REFERENCE/DOCKET NUMBER: 015280-25900US
- (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (415) 543-9600
(B) TELEFAX: (415) 543-5043

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2138 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 100..1986

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGGAATTCCG GTGGCCGGCC ACTCCCGTCT GCTGTGACGC GCGGACAGAG AGCTACCGGT 60

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Met Ala Leu Gln Arg
1 5

CTC GAC CCC TGT TGG TCC TGT GGG GAC CGC CCT GGC AGC CTC CTG TTC 162
Leu Asp Pro Cys Trp Ser Cys Gly Asp Arg Pro Gly Ser Leu Leu Phe
10 15 20

CTG CTC TTC AGC CTC GGA TGG GTG CAT CCC GCG AGG ACC CTG GCT GGA 210
Leu Leu Phe Ser Leu Gly Trp Val His Pro Ala Arg Thr Leu Ala Gly
25 30 35

GAG ACA GGG ACG GAG TCT GCC CCC CTG GGG GGA GTC CTG ACA ACC CCC 258
Glu Thr Gly Thr Glu Ser Ala Pro Leu Gly Gly Val Leu Thr Thr Pro
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CAT AAC ATT TCC AGC CTC TCC CCT CGC CAA CTC CTT GGC TTC CCG TGT 306
His Asn Ile Ser Ser Leu Ser Pro Arg Gln Leu Leu Gly Phe Pro Cys
55 60 65

GCG GAG GTG TCC GGC CTG AGC ACG GAG CGT GTC CGG GAG CTG GCT GTG 354
Ala Glu Val Ser Gly Leu Ser Thr Glu Arg Val Arg Glu Leu Ala Val
70 75 80 85

GCC TTG GCA CAG AAG AAT GTC AAG CTC TCA ACA GAG CAG CTG CGC TGT 402
Ala Leu Ala Gln Lys Asn Val Lys Leu Ser Thr Glu Gln Leu Arg Cys
90 95 100

CTG GCT CAC CGG CTC TCT GAG CCC CCC GAG GAC CTG GAC GCC CTC CCA 450
Leu Ala His Arg Leu Ser Glu Pro Pro Glu Asp Leu Asp Ala Leu Pro
105 110 115

TTG GAC CTG CTG CTA TTC CTC AAC CCA GAT GCG TTC TCG GGG CCC CAG 498
Leu Asp Leu Leu Leu Phe Leu Asn Pro Asp Ala Phe Ser Gly Pro Gln
120 125 130

GCC TGC ACC CGT TTC TTC TCC CGC ATC ACG AAG GCC AAT GTG GAC CTG 546
Ala Cys Thr Arg Phe Phe Ser Arg Ile Thr Lys Ala Asn Val Asp Leu
135 140 145

CTC CCG AGG GGG GCT CCC GAG CGA CAG CGG CTG CTG CCT GCG GCT CTG 594
Leu Pro Arg Gly Ala Pro Glu Arg Gln Arg Leu Leu Pro Ala Ala Leu
150 155 160 165

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Ala Cys Trp Gly Val Arg Gly Ser Leu Leu Ser Glu Ala Asp Val Arg
170 175 180

GCT CTG GGA GGC CTG GCT TGC GAC CTG CCT GGG CGC TTT GTG GCC GAG 690
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185 190 195

TCG GCC GAA GTG CTG CTA CCC CGG CTG GTG AGC TGC CCG GGA CCC CTG 738
Ser Ala Glu Val Leu Leu Pro Arg Leu Val Ser Cys Pro Gly Pro Leu
200 205 210

GAC CAG GAC CAG CAG GAG GCA GCC AGG GCG GCT CTG CAG GGC GGG GGA 786
Asp Gln Asp Gln Gln Glu Ala Ala Arg Ala Ala Leu Gln Gly Gly Gly
215 220 225

CCC CCC TAC GGC CCC CCG TCG ACA TGG TCT GTC TCC ACG ATG GAC GCT 834
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TCC TGG CCG CAG CCT GAA CGG ACC ATC CTC CGG CCG CGG TTC CGG CGG Ser Trp Arg Gln Pro Glu Arg Thr Ile Leu Arg Pro Arg Phe Arg Arg 280 285 290	978
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TAC CCA CAA GGT TAC CCC GAG TCT GTG ATC CAG CAC CTG GGC TAC CTC Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln His Leu Gly Tyr Leu 360 365 370	1218
TTC CTC AAG ATG AGC CCT GAG GAC ATT CGC AAG TGG AAT GTG ACG TCC Phe Leu Lys Met Ser Pro Glu Asp Ile Arg Lys Trp Asn Val Thr Ser 375 380 385	1266
CTG GAG ACC CTG AAG GCT TTG CTT GAA GTC GAC AAA GGG CAC GAA ATG Leu Glu Thr Leu Lys Ala Leu Leu Glu Val Asp Lys Gly His Glu Met 390 395 400 405	1314
AGT CCT CAG GCT CCT CGG CGG CCC CTC CCA CAG GTG GCC ACC CTG ATC Ser Pro Gln Ala Pro Arg Arg Pro Leu Pro Gln Val Ala Thr Leu Ile 410 415 420	1362
GAC CGC TTT GTG AAG GGA AGG GGC CAG CTA GAC AAA GAC ACC CTA GAC Asp Arg Phe Val Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp 425 430 435	1410
ACC CTG ACC GCC TTC TAC CCT GGG TAC CTG TGC TCC CTC AGC CCC GAG Thr Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu 440 445 450	1458
GAG CTG AGC TCC GTG CCC CCC AGC AGC ATC TGG GCG GTC AGG CCC CAG Glu Leu Ser Ser Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln 455 460 465	1506
GAC CTG GAC ACG TGT GAC CCA AGG CAG CTG GAC GTC CTC TAT CCC AAG Asp Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys 470 475 480 485	1554
GCC CGC CTT GCT TTC CAG AAC ATG AAC GGG TCC GAA TAC TTC GTG AAG Ala Arg Leu Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys 490 495 500	1602
ATC CAG TCC TTC CTG GGT GGG GCC CCC ACG GAG GAT TTG AAG GCG CTC Ile Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu 505 510 515	1650

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 ACG GAT GCG GTG CTG CCG TTG ACT GTG GCT GAG GTG CAG AAA CTT CTG 1746
 Thr Asp Ala Val Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu
 535 540 545
 GGA CCC CAC GTG GAG GGC CTG AAG GCG GAG GAG CGG CAC CGC CCG GTG 1794
 Gly Pro His Val Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val
 550 555 560 565
 CGG GAC TGG ATC CTA CGG CAG CGG CAG GAC GAC CTG GAC ACG CTG GGG 1842
 Arg Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly
 570 575 580
 CTG GGG CTA CAG GGC GGC ATC CCC AAC GGC TAC CTG GTC CTA GAC CTC 1890
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 585 590 595
 AGC GTG CAA GAG ACC CTC TCG GGG ACG CCC TGC CTC CTA GGA CCT GGA 1938
 Ser Val Gln Glu Thr Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly
 600 605 610
 CCT GTT CTC ACC GTC CTG GCA CTG CTC CTA GCC TCC ACC CTG GCC 1983
 Pro Val Leu Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
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 TGAGGGCCCC ACTCCCTTGC TGGCCCCAGC CCTGCTGGGG ATCCCCGCCT GGCCAGGAGC 2043
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 628 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Leu Gln Arg Leu Asp Pro Cys Trp Ser Cys Gly Asp Arg Pro
 1 5 10 15
 Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp Val His Pro Ala
 20 25 30
 Arg Thr Leu Ala Gly Glu Thr Gly Thr Glu Ser Ala Pro Leu Gly Gly
 35 40 45
 Val Leu Thr Thr Pro His Asn Ile Ser Ser Leu Ser Pro Arg Gln Leu
 50 55 60
 Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu Arg Val
 65 70 75 80
 Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu Ser Thr
 85 90 95
 Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro Glu Asp
 100 105 110

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Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro Asp Ala
 115 120 125
 Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile Thr Lys
 130 135 140
 Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln Arg Leu
 145 150 155 160
 Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu Leu Ser
 165 170 175
 Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu Pro Gly
 180 185 190
 Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu Val Ser
 195 200 205
 Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg Ala Ala
 210 215 220
 Leu Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp Ser Val
 225 230 235 240
 Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly Gln Pro
 245 250 255
 Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg Gln Arg
 260 265 270
 Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile Leu Arg
 275 280 285
 Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser Gly Lys
 290 295 300
 Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys Trp Glu
 305 310 315 320
 Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg
 325 330 335
 Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu Lys His
 340 345 350
 Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln
 355 360 365
 His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile Arg Lys
 370 375 380
 Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu Val Asp
 385 390 395 400
 Lys Gly His Glu Met Ser Pro Gln Ala Pro Arg Arg Pro Leu Pro Gln
 405 410 415
 Val Ala Thr Leu Ile Asp Arg Phe Val Lys Gly Arg Gly Gln Leu Asp
 420 425 430
 Lys Asp Thr Leu Asp Thr Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys
 435 440 445
 Ser Leu Ser Pro Glu Glu Leu Ser Ser Val Pro Pro Ser Ser Ile Trp
 450 455 460

00500T " 55549950

Ala Val Arg Pro Gln Asp Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp
465 470 475 480

Val Leu Tyr Pro Lys Ala Arg Leu Ala Phe Gln Asn Met Asn Gly Ser
485 490 495

Glu Tyr Phe Val Lys Ile Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu
500 505 510

Asp Leu Lys Ala Leu Ser Gln Gln Asn Val Ser Met Asp Leu Ala Thr
515 520 525

Phe Met Lys Leu Arg Thr Asp Ala Val Leu Pro Leu Thr Val Ala Glu
530 535 540

Val Gln Lys Leu Leu Gly Pro His Val Glu Gly Leu Lys Ala Glu Glu
545 550 555 560

Arg His Arg Pro Val Arg Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp
565 570 575

Leu Asp Thr Leu Gly Leu Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr
580 585 590

Leu Val Leu Asp Leu Ser Val Gln Glu Thr Leu Ser Gly Thr Pro Cys
595 600 605

Leu Leu Gly Pro Gly Pro Val Leu Thr Val Leu Ala Leu Leu Leu Ala
610 615 620

Ser Thr Leu Ala
625

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gly Gly Gly Gly Ser
1 5

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Glu Asp Leu Lys
1 5

005001"55548360

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Arg Glu Asp Leu
1

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Arg Asp Glu Leu
1

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Lys Asp Glu Leu
1

005001" 6554890

WHAT IS CLAIMED IS:

1 1. A method for specifically delivering an
2 effector molecule to a tumor cell bearing a polypeptide
3 antigen of at least 10 contiguous amino acids from the
4 polypeptide sequence of SEQ. I.D. NO. 2, wherein said
5 polypeptide binds to antisera raised against the full-length
6 polypeptide of SEQ. I.D. NO. 2 as an immunogen, which has been
7 fully immunosorbed with a 40 kD polypeptide (the K1 antigen)
 attached to the cell surface of OVCAR-3 and HeLa cells
 ("mesothelin)", said method comprising:

 providing a chimeric molecule comprising said
 effector molecule attached to a targeting molecule that
 specifically binds to mesothelin; and

 contacting said tumor with said chimeric molecule;
 wherein said chimeric molecule specifically binds to a tumor
 cell.

1 2. The method of claim 1, wherein said targeting
2 molecule is an antibody to mesothelin.

1 3. The method of claim 1, wherein said tumor is an
2 ovarian tumor cell.

1 4. The method of claim 1, wherein said effector
2 molecule is selected from the group consisting of a cytotoxin,
3 a label, a radionuclide, a drug, a liposome, a ligand, and an
4 antibody.

1 5. The method of claim 1, wherein said effector
2 molecule is a *Pseudomonas* exotoxin.

1 6. A method for impairing growth of tumor cells
2 bearing a polypeptide antigen of at least 10 contiguous amino
3 acids from the polypeptide sequence of SEQ. I.D. NO. 2,
4 wherein said polypeptide binds to antisera raised against the
5 full-length polypeptide of SEQ. I.D. NO. 2 as an immunogen,
6 which has been fully immunosorbed with a 40 kD polypeptide

(the K1 antigen) attached to the cell surface of OVCAR-3 and HeLa cells ("mesothelin"), said method comprising contacting said tumor with a chimeric molecule comprising:

a targeting molecule that specifically binds mesothelin; and

an effector molecule selected from the group consisting of a cytotoxin, a radionuclide, a ligand and an antibody; wherein said chimeric molecule specifically binds to a tumor cell.

7. The method of claim 6, wherein said cytotoxin is selected from the group consisting of *Pseudomonas* exotoxin, ricin, abrin and *Diphtheria* toxin.

8. The method of claim 6, wherein said tumor cell growth is tumor cell growth in a human.

9. The method of claim 6, wherein said contacting comprises administering said chimeric molecule to the human intravenously, into a body cavity, or into a lumen or an organ.

10. A method for detecting the presence or absence of a tumor bearing a polypeptide of at least 10 contiguous amino acids from the polypeptide sequence of SEQ. I.D. NO. 2, wherein said polypeptide binds to antisera raised against the full-length polypeptide of SEQ. I.D. NO. 2 as an immunogen, which has been fully immunosorbed with a 40 kD polypeptide (the K1 antigen) attached to the cell surface of OVCAR-3 and HeLa cells ("mesothelin"), said method comprising contacting said tumor with a chimeric molecule comprising:

a targeting molecule that specifically binds mesothelin; and a detectable label; and detecting the presence or absence of said label.

11. A pharmacological composition comprising a pharmaceutically acceptable carrier and a targeting molecule that specifically binds to a polypeptide of at least 10

contiguous amino acids from the polypeptide sequence of SEQ. I.D. NO. 2, wherein said polypeptide binds to antisera raised against the full-length polypeptide of SEQ. I.D. NO. 2 as an immunogen, which has been fully immunosorbed with a 40 kD polypeptide attached to the cell surface of OVCAR-3 and HeLa cells (the K1 antigen) in a therapeutically effective dose.

12. The pharmacological composition of claim 11, wherein the targeting molecule is further joined to an effector molecule to form a chimeric molecule.

13. The composition of claim 12, wherein said effector molecule is selected from the group consisting of a cytotoxin, a label, a radionuclide, a drug, a liposome, a ligand, and an antibody.

14. The composition of claim 12, wherein the chimeric molecule is a single-chain fusion protein.

15. A kit for the detection of mesothelin comprising a container having a nucleic acid or an antibody specific for a polypeptide of at least 10 contiguous amino acids from the polypeptide sequence of SEQ. I.D. NO. 2, wherein said polypeptide binds to antisera raised against the full-length polypeptide of SEQ. I.D. NO. 2 as an immunogen, which has been fully immunosorbed with a 40 kD polypeptide (the K1 antigen) attached to the cell surface of OVCAR-3 and HeLa cells ("mesothelin") and instructional material for the detection of tumor cells bearing mesothelin.

16. A method for the inhibition of mesothelin expression or activity comprising contacting mesothelin bearing cells with inhibitory nucleic acids specific for the nucleic acid sequence set out in SEQ. I.D. NO. 1.

17. A vaccine for the inhibition or prevention of mesotheliomas or ovarian tumors comprising administering a mesothelin derived antigen to a patient.

1 18. A method to screen drugs for treatment of tumor
2 cells bearing mesothelin comprising subjecting a mammal
3 containing transplanted tumor cells transfected with a nucleic
4 acid sequence substantially identical to a sequence of SEQ ID
5 No. 1 to a drug of interest, and monitoring tumor cell
6 activity.

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MESOTHELIN, A DIFFERENTIATION ANTIGEN PRESENT ON
MESOTHELIUM, MESOTHELIOMAS AND OVARIAN CANCERS
AND METHODS AND KITS FOR TARGETING THE ANTIGEN

5

ABSTRACT OF THE DISCLOSURE

10 This invention relates to the discovery of a
differentiation antigen termed mesothelin which is associated
with mesotheliomas and ovarian cancers. Mesothelin is about
69 kD in its full-length form. The invention includes uses
for the amino acid and nucleic acid sequences for mesothelin,
recombinant cells expressing it, methods for targeting and/or
inhibiting the growth of cells bearing mesothelin, methods for
detecting the antigen and its expression level as an
indication of the presence of tumor cells, and kits for such
15 detection.

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"Express Mail" Label No. EL623999082US

Date of Deposit October 5, 2000

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

PATENT
Attorney Docket No.: 15280-259120US
Client Reference No.: E-002-96/2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Ira Pastan and Kai Chang

Divisional of Application No.: 09/215,035

Filed: December 17, 1998

For: MESOTHELIN, A
DIFFERENTIATION ANTIGEN
PRESENT ON MESOTHELIUM,
MESOTHELIOMAS AND OVARIAN
CANCERS AND METHODS AND KITS
FOR TARGETING THE ANTIGEN

Examiner: A. Holleran

Art Unit: 1642

LETTER TO THE OFFICIAL
DRAFTSPERSON

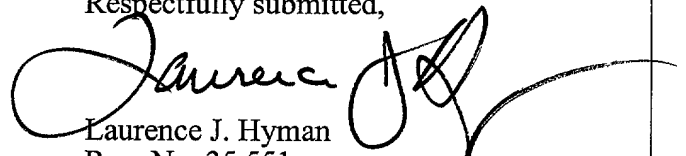
Box Patent Application

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants submit three sheets of formal drawings to be made of record in the above-identified case.

Respectfully submitted,


Laurence J. Hyman
Reg. No. 35,551

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
LJH:kad
SF 1143828 v1

005001 " 6543950

1 AGGAATTCCGGTGCCCGCCACTCCCGTCTGTGTGACCCGCGCACAGAGACTACCGGTGGACCCACCGTGCCTCCCTC
81 CCTGGGATCTACACAGACCATCGCCTTCCAACCGCTCGACCCCTGTGGTCTGTGGCGACCGCCCTCCAGCCTCCTGT
1 M A L' Q R L D P C W R C G D R P G S L L P
161 TCCTGCTCTTACGCCCTCCGATGCGTGATCCCGCAGCACCCCTGGCTGGAGACACCGACCGACTCTGCCCCCTGGGG
22 L L F S L G W V M P A R T L A G E T G T E S A P L G
241 CGACTCCTGACAAACCCCCATAACATTTCCAGCCTCTCCCTCGCCAACTCCTTGGCTTCCCGTGTGGGAGGTGCCG
48 G V L T P H N I G S L S P R Q L L G F F C A R V S G
321 CCTGAGCACGGAGCGTGTCCGGCAGCTGGCTGTGCCCTTGGCACAGAAGAATGTCAACCTCTCAACAGAGCAGCTGCGCT
73 L S T E R V R E L A V A L A Q K K V K L S T E Q L R C
401 GTCTGGCTCACCGGCTCTGTGACCCCCCAGGACCTGGACGCCCTCCCATTTGGACCTGCTCCTATTCTCAACCCAGAT
102 L A N R L S E P P E D L D A L P L D L L L F L N P D
481 GCGTTCTCGGGCCCCAGGCCCTCCACCCGTTTCTTCTCCCCCATCACGAAGGCCAATGTGACCTGCTCCGAGGGCCCC
128 A F S C P Q A C T R F F S R I T K A N V D L L P R G A
561 TCCGAGCGACAGCGGTGCTGCCTCCGCCTCTGGCCTGTGGGTGTGGGGGTCTCTCCTGAGCGAGCCTGATCTGC
155 P E R Q R L L P A A L A C W G V R G S L L S E A D V R
641 GGGCTCTGGAGGCCTGGCTTCCGACCTGCCTGGCGCTTTGTGGCCGAGTCGGCCGAAAGTGTGCTACCCCGGCTGGTG
182 A L G G L A C D L P G R P V A E S A E V L L P R L V
721 AGTGCCCGGACCCCTGGACCAGGACCGAGGAGGCCAGGCCCGCTCTGCACCCGGGGGACCCCTACGGCCC
208 S C P G P L D Q D Q Q E A A R A A L Q C G G P F Y G P
801 CCGTCGACATGGTCTCTCCACGATGGACCCCTCTGCCGGGCTGTGCTGCCCGTGTCTCCGCCAGCCCATCATCCCCAGCA
235 P S T W S V S T M D A L R G L L P V L G Q P I I R S I
881 TCCCGCAGGCCATCGTGCCGCGTGCGGCAACGCTCCTCTCGGGACCCATCCTGGCGGACGCTGAACGGACCATCCTC
262 P Q G I V A A W R Q R S S R D P S W R Q P E R T I L
961 CGCCCGCGGTTCGCCGCAAGTGGAAGADAGCCTGTCTCCTCAGGCAAGAAGCCCCGCGAGATAGACGAGCCTCAT
288 R F R F R E V E K T A C P S G K K A R E I D E S L I
1041 CTTCTACAAAGAACTGGGACCTGGAAGCCTGCGTGTGATGCGGCCCTGTGGCCACCCAGATGGACCCGCTGAACGCCATCC
315 F Y K K W E L E A C V D A A L L A T Q M D R V N A I P

FIG. 1-1.

1121 CCTTACCTACGAGCACCTGGACGTCCTAAAGCATAAACTGGATGAGCTCTACCCACAAGTTACCCCCAGTCTGTGATC
 342 F T Y E Q L D V L K H K L D E L T P Q G Y P E S V I
 1201 CAGCACCTGGGCTACCTTCTCAAGATCAGCCCTGAGGACATTCGCAAGTGAATGTGACCTCCCTCGAGACCCCTGAA
 368 Q M L G T L F L K M S P E D I E K W N V T E L R T L K
 1281 GGCTTTGCTTGAAGTCGACAAAGCCACGAAATCACTCCTCAGCCTCCTCGGGCCCCCTCCACAGGTCGCCACCCCTGA
 395 A L L E V D K G M E M S P Q A P R R P L P Q V A T L I
 1361 TCGACCCGCTTTGTGAAGGGAAGGGCCAGCTAGACAAAGACACCCCTAGACACCCCTGACCCCTTCTACCTGGGTACCTG
 422 D R F V K G R G Q L D K D T L D T L T K P Y P G Y L
 1441 TGCTCCCTCAGCCCCGAGGAGCTGACCTCCGTGCCCCCAGCACCATCTGGCGGTGTCAGGCCCCCAGGACCTGGACACGTG
 448 C S L S P E E L S S V P P S S I W A V R P Q D L D T C
 1521 TGACCCAAAGCAGCTGGACCTCCTCTATCCCAAGGCCCCCTTGCTTCCAGAACATGAACCCGTCGGAATACTTCGTGA
 475 D F Q L D V L Y P K A R L A F Q N M K G S E Y P V K
 1601 AGATCCAGTCCTTCCTGGGTGGGGCCCCCAGGAGGATTTGAAGCCGCTCAGTCAGCAGAATGTGAGCATGGACTTCGCC
 502 I Q S P L G G A P T E D L K A L S Q Q N V S M D L A
 1681 ACGTTCATGAAGCTGCGGACGGATCCGGTCCTGCCGTTGACTGTGGCTGAGGTGCAGAAACTTCTGGGACCCACGTGGA
 528 T F M K L R T D A V L P L T V A E V Q K L L C D M V E
 1761 GGGCCTGAAGCGGAGGCGCACCCCGGTGCGGACTCGATCCTACGGCAGCGGACGACGACCTGGACACGCTGG
 555 G L K A E E R H R P V R D W I L R Q R Q D D L D T L G
 1841 GGCTGGGCTACAGGGCGGCATCCCCAACGGCTACCTGCTCCTAGACCTCAGCGTGCAAGACACCCCTCTCGGGGACGCC
 582 L G L Q G G I F N C Y L V L D L S V Q E T L S C T P
 1921 TGCCTCCTAGGACCTGGACCTGTTCTCACCGTCCTGGCACTGCTCCTTAGCCTCCACCCCTGGCCTGAGGGCCCCACTCCCT
 610 C L L C P G P V L T V L A L L A S T L A
 2001 TCCTCCCCCAGCCCTCCTCGGGATCCCCCGCTGCCACACCACCGGCGGTGATCCCGTTCACCCCCAAGAGAACTC
 2081 GCCCTCAGTAAACGGGAACATGCCCCCTGCAGACAAAAAATAAAAAAAAAAAAAA 2138

FIG. 1-2.

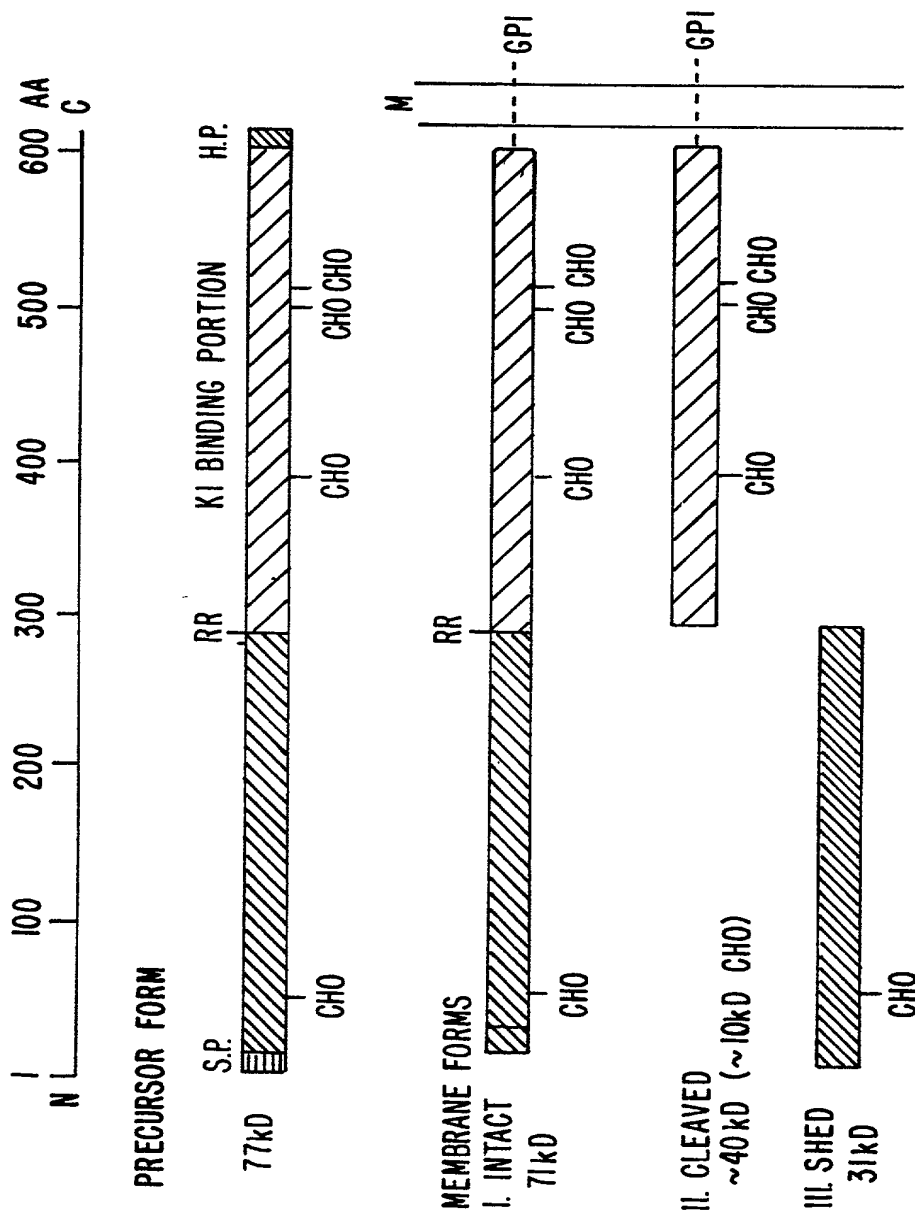


FIG. 2.

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **MESOTHELIN, A DIFFERENTIATION ANTIGEN PRESENT ON MESOTHELIUM, MESOTHELIOMAS AND OVARIAN CANCERS AND METHODS AND KITS FOR TARGETING THE ANTIGEN** the specification of which is attached hereto or X was filed on 1/21/97 as Application No. 08/776,271 and was amended on (if applicable).

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56. I claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Country	Application No.	Date of Filing	Priority Claimed Under 35 USC 119
PCT		1/3/97	Yes x No
			Yes No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date
60/010,166	1/5/96

I claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date of Filing	Status
		Patented Pending Abandoned
		Patented Pending Abandoned
		Patented Pending Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) of the Government of the United States of America as represented by the Secretary of the Department of Health and Human Services, Office of Technology Transfer, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Robert Benson, Reg. No. 33,612
Steven M. Ferguson, Reg. No. 38,448
James C. Haight, Reg. No. 25,588
Laurence J. Hyman, Reg. No. 35,551

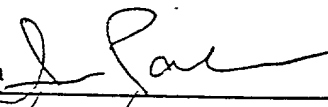
Gloria H. Richmond, Reg. No. 30,416
Susan S. Rucker, Reg. No. 35,762
David R. Sadowski, Reg. No. 32,808
Jack Spiegel, Reg. No. 34,477

Send Correspondence to Appointed Associate Attorney or Agent Address: Ellen Lauver Weber, Esq. TOWNSEND and TOWNSEND and CREW LLP Two Embarcadero Center, 8th Floor San Francisco, CA 94111-3834	Direct Telephone Calls and Facsimiles to Appointed Associate Attorney or Agent: Name: Ellen Lauver Weber, Esq. Reg. No. 32,762 Telephone: 415/576-0200 Fax: 415/576-0300
---	--

Full Name of Inventor 1	Last Name Pastan	First Name Ira	Middle Name or Initial	
Residence & Citizenship	City Potomac	State/Foreign Country Maryland	Country of Citizenship USA	
Post Office Address	Post Office Address 11710 Beall Mountain Rd.	City Potomac	State/Country Maryland	Zip Code 20854
Full Name of Inventor 2	Last Name Chang	First Name Kai	Middle Name or Initial	
Residence & Citizenship	City	State/Foreign Country	Country of Citizenship	
Post Office Address	Post Office Address	City	State/Country	Zip Code
Full Name of Inventor 3	Last Name	First Name	Middle Name or Initial	
Residence & Citizenship	City	State/Foreign Country	Country of Citizenship	
Post Office Address	Post Office Address	City	State/Country	Zip Code
Full Name of Inventor 4	Last Name	First Name	Middle Name or Initial	
Residence & Citizenship	City	State/Foreign Country	Country of Citizenship	
Post Office Address	Post Office Address	City	State/Country	Zip Code
Full Name of Inventor 5	Last Name	First Name	Middle Name or Initial	
Residence & Citizenship	City	State/Foreign Country	Country of Citizenship	
Post Office Address	Post Office Address	City	State/Country	Zip Code

Full Name of Inventor 6	Last Name	First Name	Middle Name or Initial	
Residence & Citizenship	City	State/Foreign Country	Country of Citizenship	
Post Office Address	Post Office Address	City	State/Country	Zip Code
Full Name of Inventor 7	Last Name	First Name	Middle Name or Initial	
Residence & Citizenship	City	State/Foreign Country	Country of Citizenship	
Post Office Address	Post Office Address	City	State/Country	Zip Code

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18 of the United States Code, Section 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1  Ira Pastan Date 3/19/97	Signature of Inventor 2 Kai Chang Date	Signature of Inventor 3 Date
Signature of Inventor 4	Signature of Inventor 5	Signature of Inventor 6
Date	Date	Date
Signature of Inventor 7		
Date		

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **MESOTHELIN, A DIFFERENTIATION ANTIGEN PRESENT ON MESOTHELIUM, MESOTHELIOMAS AND OVARIAN CANCERS AND METHODS AND KITS FOR TARGETING THE ANTIGEN** the specification of which is attached hereto or X was filed on 1/21/97 as Application No. and was amended on (if applicable).

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56. I claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Country	Application No.	Date of Filing	Priority Claimed Under 35 USC 119	
PCT		1/3/97	Yes x	No
			Yes	No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date
60/010,166	1/5/96

I claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date of Filing	Status		
		Patented	Pending	Abandoned
		Patented	Pending	Abandoned
		Patented	Pending	Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) of the Government of the United States of America as represented by the Secretary of the Department of Health and Human Services, Office of Technology Transfer, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Robert Benson, Reg. No. 33,612
 Steven M. Ferguson, Reg. No. 38,448
 James C. Haight, Reg. No. 25,588
 Laurence J. Hyman, Reg. No. 35,551


Gloria H. Richmond, Reg. No. 30,416
 Susan S. Rucker, Reg. No. 35,762
 David R. Sadowski, Reg. No. 32,808
 Jack Spiegel, Reg. No. 34,477

Send Correspondence to Appointed Associate Attorney or Agent Address: Ellen Lauver Weber, Esq. TOWNSEND and TOWNSEND and CREW LLP Two Embarcadero Center, 8th Floor San Francisco, CA 94111-3834	Direct Telephone Calls and Facsimiles to Appointed Associate Attorney or Agent: Name: Ellen Lauver Weber, Esq. Reg. No. 32,762 Telephone: 415/576-0200 Fax: 415/576-0300
---	--

Full Name of Inventor 1	Last Name Pastan	First Name Ira	Middle Name or Initial	
Residence & Citizenship	City Potomac	State/Foreign Country Maryland	Country of Citizenship USA	
Post Office Address	Post Office Address 11710 Beall Mountain Rd.	City Potomac	State/Country Maryland	Zip Code 20854
Full Name of Inventor 2	Last Name Chang	First Name Kai	Middle Name or Initial	
Residence & Citizenship	City <i>13223 Black Walnut Ct.</i>	State/Foreign Country <i>Silver Spring</i>	Country of Citizenship <i>P. R. CHINA</i>	
Post Office Address	Post Office Address <i>13223 Black Walnut Ct.</i>	City <i>Silver Spring</i>	State/Country <i>Maryland</i>	Zip Code <i>20906</i>
Full Name of Inventor 3	Last Name	First Name	Middle Name or Initial	
Residence & Citizenship	City	State/Foreign Country	Country of Citizenship	
Post Office Address	Post Office Address	City	State/Country	Zip Code
Full Name of Inventor 4	Last Name	First Name	Middle Name or Initial	
Residence & Citizenship	City	State/Foreign Country	Country of Citizenship	
Post Office Address	Post Office Address	City	State/Country	Zip Code
Full Name of Inventor 5	Last Name	First Name	Middle Name or Initial	
Residence & Citizenship	City	State/Foreign Country	Country of Citizenship	
Post Office Address	Post Office Address	City	State/Country	Zip Code

Full Name of Inventor 6	Last Name	First Name	Middle Name or Initial	
Residence & Citizenship	City	State/Foreign Country	Country of Citizenship	
Post Office Address	Post Office Address	City	State/Country	Zip Code
Full Name of Inventor 7	Last Name	First Name	Middle Name or Initial	
Residence & Citizenship	City	State/Foreign Country	Country of Citizenship	
Post Office Address	Post Office Address	City	State/Country	Zip Code

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18 of the United States Code, Section 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1 Ira Pastan	Signature of Inventor 2  Kai Chang	Signature of Inventor 3
Date	Date 2/16/97	Date
Signature of Inventor 4	Signature of Inventor 5	Signature of Inventor 6
Date	Date	Date
Signature of Inventor 7		
Date		

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MESOTHELIN, A DIFFERENTIATION ANTIGEN PRESENT ON MESOTHELIUM, MESOTHELIOMAS
AND OVARIAN CANCERS AND METHODS AND KITS FOR TARGETING THE ANTIGEN

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Serial No. _____

on _____

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number _____ not yet assigned

on _____ January 3, 1997

and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119	
US	60/010,166	January 5, 1996	<input checked="" type="checkbox"/> YES	<input type="checkbox"/> NO
			<input type="checkbox"/> YES	<input type="checkbox"/> NO
			<input type="checkbox"/> YES	<input type="checkbox"/> NO
			<input type="checkbox"/> YES	<input type="checkbox"/> NO
			<input type="checkbox"/> YES	<input type="checkbox"/> NO

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS (Check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED
PCT APPLICATIONS DESIGNATING THE U.S.				
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)		

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (List name and registration number)

Guy W. Chambers, Reg. No. 30,617
Kenneth A. Weber, Reg. No. 31,677
Ellen L. Weber, Reg. No. 32,762

Correspondence to: Ellen L. Weber
ownsend and Townsend and Crew LLP
wo Embarcadero Center, 8th Fl.
an Francisco, CA 94111-3834

Direct Telephone Calls to:
(name and telephone number)
Ellen L. Weber
415-576-0200

FULL NAME OF INVENTOR	FAMILY NAME PASTAN	FIRST GIVEN NAME Ira	SECOND GIVEN NAME
RESIDENCE & CITIZENSHIP	CITY Potomac	STATE OR FOREIGN COUNTRY Maryland	COUNTRY OF CITIZENSHIP USA
POST OFFICE ADDRESS	POST OFFICE ADDRESS 11710 Beal Mountain Road	CITY Potomac	STATE & ZIP CODE/COUNTRY Maryland 20854
FULL NAME OF INVENTOR	FAMILY NAME CHANG	FIRST GIVEN NAME Kai	SECOND GIVEN NAME
RESIDENCE & CITIZENSHIP	CITY Silver Spring	STATE OR FOREIGN COUNTRY Maryland	COUNTRY OF CITIZENSHIP China
POST OFFICE ADDRESS	POST OFFICE ADDRESS 13223 Black Walnut Court	CITY Silver Spring	STATE & ZIP CODE/COUNTRY Maryland 20906
FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

NATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
TE	DATE	DATE

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Pastan, Ira
Chang, Kai
- (ii) TITLE OF INVENTION: Mesothelin, a Differentiation Antigen
Present on Mesothelium, Mesotheliomas and Ovarian Cancers
and Methods and Kits for Targeting the Antigen
- (iii) NUMBER OF SEQUENCES: 8
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Townsend and Townsend and Crew LLP
(B) STREET: Two Embarcadero Center, Eighth Floor
(C) CITY: San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94111-3834
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Not yet assigned
(B) FILING DATE: Not yet assigned
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/776,271
(B) FILING DATE: 01-DEC-1998
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: WO PCT/US97/00224
(B) FILING DATE: 03-JAN-1997
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/010,166
(B) FILING DATE: 05-JAN-1996
- (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Faris, Susan K.
(B) REGISTRATION NUMBER: 41,739
(C) REFERENCE/DOCKET NUMBER: 015280-259110US
- (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (415) 576-0200
(B) TELEFAX: (415) 576-0300

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2138 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 100..1986

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AGGAATTCCG GTGGCCGGCC ACTCCCGTCT GCTGTGACGC GCGGACAGAG AGCTACCGGT      60
GGACCCACGG TGCCTCCCTC CCTGGGATCT ACACAGACC ATG GCC TTG CAA CGG      114
                               Met Ala Leu Gln Arg
                               1             5
CTC GAC CCC TGT TGG TCC TGT GGG GAC CGC CCT GGC AGC CTC CTG TTC      162
Leu Asp Pro Cys Trp Ser Cys Gly Asp Arg Pro Gly Ser Leu Leu Phe
                               10             15             20
CTG CTC TTC AGC CTC GGA TGG GTG CAT CCC GCG AGG ACC CTG GCT GGA      210
Leu Leu Phe Ser Leu Gly Trp Val His Pro Ala Arg Thr Leu Ala Gly
                               25             30             35
GAG ACA GGG ACG GAG TCT GCC CCC CTG GGG GGA GTC CTG ACA ACC CCC      258
Glu Thr Gly Thr Glu Ser Ala Pro Leu Gly Gly Val Leu Thr Thr Pro
                               40             45             50
CAT AAC ATT TCC AGC CTC TCC CCT CGC CAA CTC CTT GGC TTC CCG TGT      306
His Asn Ile Ser Ser Leu Ser Pro Arg Gln Leu Leu Gly Phe Pro Cys
                               55             60             65
GCG GAG GTG TCC GGC CTG AGC ACG GAG CGT GTC CGG GAG CTG GCT GTG      354
Ala Glu Val Ser Gly Leu Ser Thr Glu Arg Val Arg Glu Leu Ala Val
                               70             75             80             85
GCC TTG GCA CAG AAG AAT GTC AAG CTC TCA ACA GAG CAG CTG CGC TGT      402
Ala Leu Ala Gln Lys Asn Val Lys Leu Ser Thr Glu Gln Leu Arg Cys
                               90             95             100
CTG GCT CAC CGG CTC TCT GAG CCC CCC GAG GAC CTG GAC GCC CTC CCA      450
Leu Ala His Arg Leu Ser Glu Pro Pro Glu Asp Leu Asp Ala Leu Pro
                               105            110            115
TTG GAC CTG CTG CTA TTC CTC AAC CCA GAT GCG TTC TCG GGG CCC CAG      498
Leu Asp Leu Leu Leu Phe Leu Asn Pro Asp Ala Phe Ser Gly Pro Gln
                               120            125            130
GCC TGC ACC CGT TTC TTC TCC CGC ATC ACG AAG GCC AAT GTG GAC CTG      546
Ala Cys Thr Arg Phe Phe Ser Arg Ile Thr Lys Ala Asn Val Asp Leu
                               135            140            145

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CC50T 554350

CTC	CCG	AGG	GGG	GCT	CCC	GAG	CGA	CAG	CGG	CTG	CTG	CCT	GCG	GCT	CTG	594
Leu	Pro	Arg	Gly	Ala	Pro	Glu	Arg	Gln	Arg	Leu	Leu	Pro	Ala	Ala	Leu	
150					155					160					165	
GCC	TGC	TGG	GGT	GTG	CGG	GGG	TCT	CTG	CTG	AGC	GAG	GCT	GAT	GTG	CGG	642
Ala	Cys	Trp	Gly	Val	Arg	Gly	Ser	Leu	Leu	Ser	Glu	Ala	Asp	Val	Arg	
				170					175					180		
GCT	CTG	GGA	GGC	CTG	GCT	TGC	GAC	CTG	CCT	GGG	CGC	TTT	GTG	GCC	GAG	690
Ala	Leu	Gly	Gly	Leu	Ala	Cys	Asp	Leu	Pro	Gly	Arg	Phe	Val	Ala	Glu	
			185					190					195			
TCG	GCC	GAA	GTG	CTG	CTA	CCC	CGG	CTG	GTG	AGC	TGC	CCG	GGA	CCC	CTG	738
Ser	Ala	Glu	Val	Leu	Leu	Pro	Arg	Leu	Val	Ser	Cys	Pro	Gly	Pro	Leu	
		200					205					210				
GAC	CAG	GAC	CAG	CAG	GAG	GCA	GCC	AGG	GCG	GCT	CTG	CAG	GGC	GGG	GGA	786
Asp	Gln	Asp	Gln	Gln	Glu	Ala	Ala	Arg	Ala	Ala	Leu	Gln	Gly	Gly	Gly	
	215					220					225					
CCC	CCC	TAC	GGC	CCC	CCG	TCG	ACA	TGG	TCT	GTC	TCC	ACG	ATG	GAC	GCT	834
Pro	Pro	Tyr	Gly	Pro	Pro	Ser	Thr	Trp	Ser	Val	Ser	Thr	Met	Asp	Ala	
230					235					240					245	
CTG	CGG	GGC	CTG	CTG	CCC	GTG	CTG	GGC	CAG	CCC	ATC	ATC	CGC	AGC	ATC	882
Leu	Arg	Gly	Leu	Leu	Pro	Val	Leu	Gly	Gln	Pro	Ile	Ile	Arg	Ser	Ile	
				250					255					260		
CCG	CAG	GGC	ATC	GTG	GCC	GCG	TGG	CGG	CAA	CGC	TCC	TCT	CGG	GAC	CCA	930
Pro	Gln	Gly	Ile	Val	Ala	Ala	Trp	Arg	Gln	Arg	Ser	Ser	Arg	Asp	Pro	
			265					270					275			
TCC	TGG	CGG	CAG	CCT	GAA	CGG	ACC	ATC	CTC	CGG	CCG	CGG	TTC	CGG	CGG	978
Ser	Trp	Arg	Gln	Pro	Glu	Arg	Thr	Ile	Leu	Arg	Pro	Arg	Phe	Arg	Arg	
		280					285					290				
GAA	GTG	GAG	AAG	ACA	GCC	TGT	CCT	TCA	GGC	AAG	AAG	GCC	CGC	GAG	ATA	1026
Glu	Val	Glu	Lys	Thr	Ala	Cys	Pro	Ser	Gly	Lys	Lys	Ala	Arg	Glu	Ile	
	295					300					305					
GAC	GAG	AGC	CTC	ATC	TTC	TAC	AAG	AAG	TGG	GAG	CTG	GAA	GCC	TGC	GTG	1074
Asp	Glu	Ser	Leu	Ile	Phe	Tyr	Lys	Lys	Trp	Glu	Leu	Glu	Ala	Cys	Val	
310					315					320					325	
GAT	GCG	GCC	CTG	CTG	GCC	ACC	CAG	ATG	GAC	CGC	GTG	AAC	GCC	ATC	CCC	1122
Asp	Ala	Ala	Leu	Leu	Ala	Thr	Gln	Met	Asp	Arg	Val	Asn	Ala	Ile	Pro	
				330					335					340		
TTC	ACC	TAC	GAG	CAG	CTG	GAC	GTC	CTA	AAG	CAT	AAA	CTG	GAT	GAG	CTC	1170
Phe	Thr	Tyr	Glu	Gln	Leu	Asp	Val	Leu	Lys	His	Lys	Leu	Asp	Glu	Leu	
			345					350					355			
TAC	CCA	CAA	GGT	TAC	CCC	GAG	TCT	GTG	ATC	CAG	CAC	CTG	GGC	TAC	CTC	1218
Tyr	Pro	Gln	Gly	Tyr	Pro	Glu	Ser	Val	Ile	Gln	His	Leu	Gly	Tyr	Leu	
		360					365					370				
TTC	CTC	AAG	ATG	AGC	CCT	GAG	GAC	ATT	CGC	AAG	TGG	AAT	GTG	ACG	TCC	1266
Phe	Leu	Lys	Met	Ser	Pro	Glu	Asp	Ile	Arg	Lys	Trp	Asn	Val	Thr	Ser	
	375					380					385					

00500T " 55543360

CTG GAG ACC CTG AAG GCT TTG CTT GAA GTC GAC AAA GGG CAC GAA ATG 1314
 Leu Glu Thr Leu Lys Ala Leu Leu Glu Val Asp Lys Gly His Glu Met
 390 395 400 405

AGT CCT CAG GCT CCT CGG CGG CCC CTC CCA CAG GTG GCC ACC CTG ATC 1362
 Ser Pro Gln Ala Pro Arg Arg Pro Leu Pro Gln Val Ala Thr Leu Ile
 410 415 420

GAC CGC TTT GTG AAG GGA AGG GGC CAG CTA GAC AAA GAC ACC CTA GAC 1410
 Asp Arg Phe Val Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp
 425 430 435

ACC CTG ACC GCC TTC TAC CCT GGG TAC CTG TGC TCC CTC AGC CCC GAG 1458
 Thr Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu
 440 445 450

GAG CTG AGC TCC GTG CCC CCC AGC AGC ATC TGG GCG GTC AGG CCC CAG 1506
 Glu Leu Ser Ser Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln
 455 460 465

GAC CTG GAC ACG TGT GAC CCA AGG CAG CTG GAC GTC CTC TAT CCC AAG 1554
 Asp Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys
 470 475 480 485

GCC CGC CTT GCT TTC CAG AAC ATG AAC GGG TCC GAA TAC TTC GTG AAG 1602
 Ala Arg Leu Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys
 490 495 500

ATC CAG TCC TTC CTG GGT GGG GCC CCC ACG GAG GAT TTG AAG GCG CTC 1650
 Ile Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu
 505 510 515

AGT CAG CAG AAT GTG AGC ATG GAC TTG GCC ACG TTC ATG AAG CTG CGG 1698
 Ser Gln Gln Asn Val Ser Met Asp Leu Ala Thr Phe Met Lys Leu Arg
 520 525 530

ACG GAT GCG GTG CTG CCG TTG ACT GTG GCT GAG GTG CAG AAA CTT CTG 1746
 Thr Asp Ala Val Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu
 535 540 545

GGA CCC CAC GTG GAG GGC CTG AAG GCG GAG GAG CGG CAC CGC CCG GTG 1794
 Gly Pro His Val Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val
 550 555 560 565

CGG GAC TGG ATC CTA CGG CAG CGG CAG GAC GAC CTG GAC ACG CTG GGG 1842
 Arg Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly
 570 575 580

CTG GGG CTA CAG GGC GGC ATC CCC AAC GGC TAC CTG GTC CTA GAC CTC 1890
 Leu Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp Leu
 585 590 595

AGC GTG CAA GAG ACC CTC TCG GGG ACG CCC TGC CTC CTA GGA CCT GGA 1938
 Ser Val Gln Glu Thr Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly
 600 605 610

CCT GTT CTC ACC GTC CTG GCA CTG CTC CTA GCC TCC ACC CTG GCC 1983
 Pro Val Leu Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
 615 620 625

TGAGGGCCCC ACTCCCTTGC TGGCCCCAGC CCTGCTGGGG ATCCCCGCCT GGCCAGGAGC 2043

09684599-10000

AGGCACGGGT	GATCCCCGTT	CCACCCCAAG	AGAACTCGCG	CTCAGTAAAC	GGGAACATGC	2103
CCCCTGCAGA	CAAAAAAAAAA	AAAAAAAAAA	AAAAA			2138

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 628 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Ala	Leu	Gln	Arg	Leu	Asp	Pro	Cys	Trp	Ser	Cys	Gly	Asp	Pro	Pro	
1					5				10					15		
Gly	Ser	Leu	Leu	Phe	Leu	Leu	Phe	Ser	Leu	Gly	Trp	Val	His	Pro	Ala	
			20					25					30			
Arg	Thr	Leu	Ala	Gly	Glu	Thr	Gly	Thr	Glu	Ser	Ala	Pro	Leu	Gly	Gly	
		35					40					45				
Val	Leu	Thr	Thr	Pro	His	Asn	Ile	Ser	Ser	Leu	Ser	Pro	Arg	Gln	Leu	
	50					55					60					
Leu	Gly	Phe	Pro	Cys	Ala	Glu	Val	Ser	Gly	Leu	Ser	Thr	Glu	Arg	Val	
65					70					75					80	
Arg	Glu	Leu	Ala	Val	Ala	Leu	Ala	Gln	Lys	Asn	Val	Lys	Leu	Ser	Thr	
				85					90					95		
Glu	Gln	Leu	Arg	Cys	Leu	Ala	His	Arg	Leu	Ser	Glu	Pro	Pro	Glu	Asp	
			100					105					110			
Leu	Asp	Ala	Leu	Pro	Leu	Asp	Leu	Leu	Leu	Phe	Leu	Asn	Pro	Asp	Ala	
		115					120					125				
Phe	Ser	Gly	Pro	Gln	Ala	Cys	Thr	Arg	Phe	Phe	Ser	Arg	Ile	Thr	Lys	
	130					135					140					
Ala	Asn	Val	Asp	Leu	Leu	Pro	Arg	Gly	Ala	Pro	Glu	Arg	Gln	Arg	Leu	
145					150					155					160	
Leu	Pro	Ala	Ala	Leu	Ala	Cys	Trp	Gly	Val	Arg	Gly	Ser	Leu	Leu	Ser	
				165					170					175		
Glu	Ala	Asp	Val	Arg	Ala	Leu	Gly	Gly	Leu	Ala	Cys	Asp	Leu	Pro	Gly	
			180					185					190			
Arg	Phe	Val	Ala	Glu	Ser	Ala	Glu	Val	Leu	Leu	Pro	Arg	Leu	Val	Ser	
		195					200					205				
Cys	Pro	Gly	Pro	Leu	Asp	Gln	Asp	Gln	Gln	Glu	Ala	Ala	Arg	Ala	Ala	
	210					215					220					
Leu	Gln	Gly	Gly	Gly	Pro	Pro	Tyr	Gly	Pro	Pro	Ser	Thr	Trp	Ser	Val	
225					230					235					240	

Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly Gln Pro
 245 250 255
 Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg Gln Arg
 260 265 270
 Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile Leu Arg
 275 280 285
 Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser Gly Lys
 290 295 300
 Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys Trp Glu
 305 310 315 320
 Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg
 325 330 335
 Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu Lys His
 340 345 350
 Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln
 355 360 365
 His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile Arg Lys
 370 375 380
 Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu Val Asp
 385 390 395 400
 Lys Gly His Glu Met Ser Pro Gln Ala Pro Arg Arg Pro Leu Pro Gln
 405 410 415
 Val Ala Thr Leu Ile Asp Arg Phe Val Lys Gly Arg Gly Gln Leu Asp
 420 425 430
 Lys Asp Thr Leu Asp Thr Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys
 435 440 445
 Ser Leu Ser Pro Glu Glu Leu Ser Ser Val Pro Pro Ser Ser Ile Trp
 450 455 460
 Ala Val Arg Pro Gln Asp Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp
 465 470 475 480
 Val Leu Tyr Pro Lys Ala Arg Leu Ala Phe Gln Asn Met Asn Gly Ser
 485 490 495
 Glu Tyr Phe Val Lys Ile Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu
 500 505 510
 Asp Leu Lys Ala Leu Ser Gln Gln Asn Val Ser Met Asp Leu Ala Thr
 515 520 525
 Phe Met Lys Leu Arg Thr Asp Ala Val Leu Pro Leu Thr Val Ala Glu
 530 535 540
 Val Gln Lys Leu Leu Gly Pro His Val Glu Gly Leu Lys Ala Glu Glu
 545 550 555 560

00500T " 555433550

Arg His Arg Pro Val Arg Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp
 565 570 575
 Leu Asp Thr Leu Gly Leu Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr
 580 585 590
 Leu Val Leu Asp Leu Ser Val Gln Glu Thr Leu Ser Gly Thr Pro Cys
 595 600 605
 Leu Leu Gly Pro Gly Pro Val Leu Thr Val Leu Ala Leu Leu Leu Ala
 610 615 620
 Ser Thr Leu Ala
 625

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gly Gly Gly Gly Ser
 1 5

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Glu Asp Leu Lys
 1 5

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

005001" 664360

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Arg Glu Asp Leu
1

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Arg Asp Glu Leu
1

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Lys Asp Glu Leu
1

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Arg Pro Arg Phe Arg Arg
1 5

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